EFFECTS OF THE GREGARINES *ASCOGREGARINA CULICIS* (ROSS) AND *ASCOGREGARINA TAIWANENSIS* (LIEN AND LEVINE) (APICOMPLEXA: LECUDINIDAE) UPON THEIR CORRESPONDING HOSTS, *AEDES AEGYPTI* (L.) AND *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE)

By

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Dedicated to
My beloved father, Lucio Reyes-Faz
Wherever you are,
And to my children: Cynthia, Coryna, Tanya and Filiberto Jr.
Who are the reason for my life

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Abstract of Dissertation Presented to the Graduate School
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EFFECTS OF THE GREGARINES *ASCOGREGARINA CULICIS* (ROSS) AND *ASCOGREGARINA TAIWANENSIS* (LIEN AND LEVINE) (APICOMPLEXA: LECUDINIDAE) UPON THEIR CORRESPONDING HOSTS, *AEDES AEGYPTI* (L.) AND *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE)

By

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Five experiments to examine the susceptibility of *Aedes aegypti* to *Ascogregarina culicis* and *Ae. albopictus* to *A. taiwanensis*, and parasite impact upon host fitness under competition, were carried out. Host and parasite strains were from Tampa, FL, where both mosquito species are sympatric. Bioassays demonstrated that *Ae. aegypti* is more susceptible to the infection by *A. culicis* than *Ae. albopictus* to *A. taiwanensis*. Pupation in either species was not affected by parasitism. After dissection of multiple dual infected larvae, the brown pigmentation and the rounded protomerite in *A. taiwanensis* gamonts distinguish them from the translucent and tapered protomerite gamonts of *A. culicis* under phase-contrast microscope. In single and mixed larval treatments exposed to single and dual infections (100 oocysts/larva), *Ae. aegypti* had a higher infection intensity than *Ae. albopictus*. Impact of two food levels (regular diet versus 75% reduced regular diet) and a high infection level (0 and 1000 oocysts/larva) were evaluated from mixed and dual infected larval treatments upon wing-length and larval development time. In the high food treatments, the wing-length in both mosquito hosts was not affected by parasitism. In the low food level, the infection decreased the wing-length of *Ae. aegypti* males but not in females, while the same effect was observed only in the *Ae. albopictus* males. Female larval development time in either *Aedes* species did not vary in function of parasitism in the high food level treatments. In contrast, in the low food treatments, the parasitism prolonged the development time of *Ae. aegypti* females but not males, while the development also was extended in *Ae. albopictus* males but not in females.

When larvae of *Ae. aegypti* were infected with *A. culicis* and *Ae. albopictus* with *A. taiwanensis* (1000 oocysts/larva) and both species were confined in the same tire in field with oak leaves, the wing-length and performance index (lambda) were not affected by parasitism, but λ' of unparasitized *Ae. aegypti* was higher under intra- than interspecific competition. *Ae. albopictus* males developed faster than infected *Ae. aegypti.* However, *A. culicis* increased the *Ae. aegypti* survivorship to adulthood in comparison with its control.

CHAPTER 1
LITERATURE REVIEW

Introduction

When *Homo sapiens* appeared on the earth, most blood-feeding insect and arthropod groups involved in the transmission of modern diseases had already evolved in association with primitive man. Certainly, these human - vector interactions had their origins with the first hominids. The everyday life of primitive man suffered the attack of populations of hungry, haematophagous arthropods several million years ago. Because of these trophic relationships, certain microorganisms living in symbiosis within arthropods became causal agents of human diseases. The triangle generated by the parasite and /or pathogen, the arthropod as vector or transmitter, and man as the host, is the cornerstone of medical and veterinary entomology, a research field that encompasses parasitology, entomology and epidemiology as important sciences.

Symbiosis is a cohesive concept in biology. It includes parasitological relationships, in which one organism obtains its energy from another in various ways. Sometimes the energy exchange between the associated organisms in symbiotic relationships is slightly unbalanced. There are no clear boundaries that define which organisms receiving the energy might be called "parasites". Direct death of the host is not required because a reduction in fitness and reproduction might cause a slow but detrimental impact upon the host population density, which could lead to its delay or disappearance in a given habitat. This may happen in gregarine parasites that attack insects. Gregarines (Apicomplexa: Lecudinidae) appear relatively harmless to their natural mosquito hosts, but there is limited information on the long-term impact on overall host population regulation (Garcia et al. 1994). At present, there are 214 genera and approximately 1,450 species of gregarines parasitizing insects (Levine 1985) and among them species of the genus *Ascogregarina* (Lecudinidae) are known to parasitize eight species of mosquitoes (Diptera: Culicidae) (Beier and Craig 1985). The genus *Ascogregarina* (Ward et al. 1982) has been changed twice since its type species *Gregarinida culicidis* was described by Sir Ronald Ross in 1895 (Beier and Craig 1985). Subsequently, the genus *Lankesteria* (Wenyon 1926) replaced Gregarinida, followed by the genus *Ascocystis*, which included only insect gregarines (Grasse 1953).

The reproduction of *Ascogregarina* is exclusively sexual wherein two mature trophozoites or gamonts living in the larval midgut migrate into the Malpighian tubules where they fuse (syzygy) to form a gametocyst (Levine 1977). The replicative phases occur in the gametocyst and sporocyst through gametogony and sporogony (Chen et al. 1997b). One gamont produces numerous male gametes while the other generates abundant female gametes, and then by fertilization an oocyst is produced (Chen and Yang 1996). Oocysts are forms that infect new hosts. They are released to the environment when mosquito females oviposit, or an adult mosquito defecates or dies in a larval developmental site (Beier and Craig 1985). Each oocyst contains eight non-segmented sporozoites, which are released within the larval midgut after ingestion (Chen et al. 1997a).

It is commonly observed in parasitology that the pathogenic effect of a parasite is less marked when the parasitic organisms are in their natural hosts compared to when they are living in hosts they do not normally inhabit (Anderson 1979). This is typical in parasitism by *Ascogregarina* spp. of *Aedes* mosquito species. Various but low pathogenicity levels have evolved in the interactions between host-specific *Ascogregarina culicis* and *Aedes aegypti*, and *A. taiwanensis* and *Aedes albopictus* (Walsh and Olson 1976, Garcia et al. 1994). These relationships are potential research tools to examine the possible role of parasitism by *Ascogregarina* spp. on the competitive interactions between these *Aedes* species, which may occur sympatrically.

Aedes aegypti and *Ae. albopictus* are important vectors of dengue (Hawley 1988), and also harbor more than five arboviruses that have been isolated from field-collected specimens in the United States (Mitchell et al. 1998, Gerhardt et al. 2001, in press). The presence of developing *Dirofilaria* sp. larvae in the Malpighian tubules of field-caught females indicate that *Ae. albopictus* may be naturally infected with dog heartworm in Florida (Nayar and Knight 1999).

An intensification of rural dengue transmission is possible because *Ae. albopictus* is known to displace to *Ae. aegypti* as has already occurred in the southeast USA (Ali and Nayar 1997). Therefore, the ecological consequences of the displacement may have a direct influence on dengue epidemiology and on the other diseases that *Ae. albopictus* is capable of transmitting. The phenomenon is complex because many environmental factors are involved and thus far, various hypotheses have been proposed to explain this displacement.

The Asian tiger mosquito *Ae. albopictus* (Skuse) was detected for the first time in the USA in 1972 (Eads 1972) and then in 1984 in Tennessee (Reiter and Darsie 1984). However, properly established populations in USA were reported in Harris County, Houston, Texas, in 1985 (Sprenger and Wuithiranyagool 1986). A year later, it was reported in Jacksonville, Florida (Peacock et al. 1988), and currently this species is established throughout Florida (Ali and Nayar 1997). To date, there are five states (Florida, Georgia, South Carolina, North Carolina, and Tennessee) in which *Ae. albopictus* has been found in all counties; the species is currently believed to be established in 911 counties and 25 states (Moore 1999).

The geographic dispersion pattern for *Ae. albopictus* in the southeast USA is related, in part, with the transportation and commerce along the interstate highway systems (Moore and Mitchell 1997). Likewise, the northern and western limits are influenced by low temperatures and dryness, and it has been speculated that it will be more difficult for *Ae. albopictus* to colonize the Pacific coast states because of the adverse climate, especially the predominance of rainfall during the cooler winter months (Moore 1999). Nevertheless, *Ae. albopictus* is capable of invading temperate regions because of its tolerance of low temperature. Temperate strains of this mosquito may survive exposure to -10°C for 24 h in egg diapause. This feature allows it to overwinter as far north as 42°N, where *Ae. aegypti* cannot survive (Hawley et al. 1987).

Aedes aegypti, which is the primary vector of the dengue viruses worldwide, is thought to have originated in East Africa (Tabachnick 1991). It became cosmopolitan in the late 1700s and was responsible for dengue and yellow fever epidemics documented in the literature. The first dengue epidemic in the USA occurred in Philadelphia in 1780 when Dr. Benjamin Rush named this disease "Breakbone fever" because of the symptom of aching joints (Chapman 1978). Nevertheless, *Ae. aegypti* has been an introduced member of the New World mosquito communities for the last 440 years because according to Tabachnick (1991), the first yellow fever epidemics occurred in Yucatan, Mexico in 1560. Because the species cannot tolerate temperatures below 48°F and will die after less than an hour of exposure to 32°F, its distribution is limited to a range below 35°N, which includes some tropical urban areas of the world (Centers for Disease Control 1979). In the USA, it can be found during summer in southeastern states, such as parts of Florida, Louisiana, and Texas, and survives in these areas in the egg stage (CDC 1998).

The *Aedes albopictus* invasion and establishment in the USA has resulted in some shifts in the mosquito communities associated with tree-holes and man-made containers (discarded tires, etc.). Consequently, almost 10 years after the *Ae. albopictus* introduction, *Ae. aegypti* populations have essentially disappeared in north Florida, declined in central Florida, and remain present at various densities in south Florida (O'Meara et al. 1995).

Interspecific competition among larvae of container-inhabiting *Aedes* mosquitoes has been intensively investigated. In general, the competition may be direct or indirect (Barrera 1996). Direct competition occurs when one species exerts a direct antagonistic effect against the other, for instance, the production of larval growth retardants by *Ae. aegypti* (Kuno and Moore 1975). It is also direct when one species becomes superior because of some feature in its biology that makes it a better competitor. This is the case with *Ae. aegypti* and *Ae. albopictus* when both are present in low larval densities and reared with artificial food in the laboratory. Under these circumstances, there is always a higher survivorship to adulthood in *Ae. aegypti* than in *Ae. albopictus* (Black et al. 1989, Ho et al. 1989). A faster ingestion rate in larvae of *Ae. aegypti* than in *Ae. albopictus* has been proposed to explain this result (Ho et al. 1989).

Indirect competition has various expressions. One is when *Ae. albopictus* populations were diminished in Singapore as a consequence of urbanization. Because *Ae. albopictus* is exophilic, when vegetated areas were eliminated in and around the city, *Ae. aegypti*, which is endophilic, was able to dominate (Chan et al. 1971).

A similar explanation based on habitat segregation and differences in domesticity has been suggested to explain the displacement of *Ae. aegypti* by *Ae. albopictus* in the USA (Barrera 1996). According to this hypothesis, *Ae. albopictus* has been invading habitats (breeding sites in vegetated areas), in which it is better adapted than *Ae. aegypti*, a species more endophagic and anthropophilic than *Ae. albopictus*. In contrast, *Ae. albopictus* is exophagic and less anthropophilic. Hence, *Ae. aegypti* vanished from vegetated and peridomestic habitats in which it survived not as well as *Ae. albopictus* (Barrera 1996).

Alternativeley, because *Ascogregarina* spp. are prevalent in wild mosquito populations, some reports have mentioned that the gregarines (Teng et al. 1996, Blackmore et al. 1995) could have a significant although non-essential role in limiting *Ae. aegypti* distribution. Presently, *Ascogregarina taiwanensis* and *A. culicis* are host-specific to *Ae. albopictus* and *Ae. aegypti*, respectively (Blackmore et al. 1995). In Texas, it has been reported that in wild larval populations of *Ae. aegypti*, the *A. culicis* infection rate reaches 86% (Barrett et al. 1971), whereas in Florida the larval infection ranged from 32 to 86% (Stapp and Casten 1971).

It has been reported that *A. culicis* may inflict significant mortality on some Asian strains of *Ae. aegypti* (Sulaiman 1992). However, *A. culicis* usually does not harm *Ae. aegypti*, but is lethal to other mosquito species, such as *Ae. epactius* and *Ae. atropalpus*

(Spencer and Olson 1982). Pathogenic effects are observed in midgut epithelial cells which are readily replaced, but more important is the destruction of the Malpighian tubule cells which have little cellular regeneration (McCray et al. 1970).

The number of *A. culicis* gamonts produced per *Aedes aegypti* larva is highly variable. There are from 1 to 2000 trophozoites per larva, and an infection intensity commonly observed is 40 to 100 trophozoites per larva (Gentile et al. 1971). In a field study, for *Ae. aegypti* populations in Houston, an infection of 250 gamonts per larva may kill larvae or decrease the larval size (Barrett 1968). An infection intensity of 8 to 25 gametocysts could destroy one third of a Malpighian tubule, and higher infection levels destroy the distal two-thirds of the five Malpighian tubules. This usually causes pupal death (Barrett 1968). However, Gentile et al. (1971) stated that no pathogenic effects were observed in larvae containing over 300 trophozoites or when Malpighian tubules were heavily infected with gametocysts.

Walsh and Olson (1976) reported a 98% larval infection rate and 11% pupal mortality infecting 100 1st instar *Ae. aegypti* with 10,000 *A. culicis* oocysts in 1 liter of water. They observed encapsulated gamonts in larval midgets and a field release of 20,000 to 36,000 oocysts per square foot of larval feeding area caused a temporary increase in the infection level of 40 – 100 trophozoites per larva.

In the Florida cities of West Palm Beach, Boca Raton, Miami and Homestead, the proportion of *Ae. aegypti* larvae infected with *A. culicis* ranged from 32 to 86% (Stapp and Casten 1971). In this study, the infection intensity varied from 1 to 800 gamonts per larva, but the mean number for the entire study was 57 gamonts per larva. In a more recent study, 23 and 29 localities were sampled for infection with *A. culicis* in *Ae.*

aegypti, and *A. taiwanensis* in *Ae. albopictus*, respectively. The range of infection intensity (1 – 486 trophozoites per larva) was not different between species, but the average number of parasites was significantly higher in *Ae. aegypti* (52.5 per host) than in *Ae. albopictus* (33.5 per host) (Blackmore et al. 1995). Under laboratory conditions, mortality in *Ae. aegypti* and *Ae. albopictus* larvae infected with *A. taiwanensis* (75 larvae exposed to 1000 oocysts per ml) was not significant (9.1 and 9.5 %) and not different from other reports (Garcia et al. 1994).

Statement of Purpose

Craig in (1993) proposed that the differential pathogenicity of gregarines could determine displacement of *Ae. aegypti* by *Ae. albopictus*. However, further research is required to examine factors such as the pathogenic effects in each host species, frequency of cross infections, and density-dependent pathogenicity (Blackmore et al. 1995).

In an attempt to evaluate Craig's hypothesis, the objectives in this study were:

1. To evaluate, under laboratory conditions, the susceptibility of *Ae. aegypti* to *A. culicis*, and of *Ae. albopictus* to *A. taiwanensis*, and to determine the impact of the parasite on pupation rates in each host/parasite system.
2. To determine, in the laboratory, the gamont intensity (gamont load/larvae) of *A. culicis* and *A. taiwanensis* in single and mixed larval populations of *Ae. aegypti* and *Ae. albopictus* exposed to single, cross-, and dual-infections.
3. To determine, under laboratory conditions, the impact of a high infection level (1000 oocysts/larva) of each gregarine species, and two larval food levels (low and standard) upon wing-length (mm) and larval development time in both sexes, for each mosquito host species.

4. To estimate, under field conditions, the effect of single and mixed larval treatments of both mosquito hosts in which each host was infected with its own gregarine (1000 oocysts/larva) upon wing-length, immature development time (from egg hatching to adult emergence), survivorship to adulthood (% of larvae surviving to adult stage), days as adult (from adult emergence to death), and a performance index (λ') the finite rate of increase, based on each *Aedes* species.

CHAPTER 2

SUSCEPTIBILITY OF *AEDES AEGYPTI* (L.) AND *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) TO THE INFECTION OF *ASCOGREGARINA CULICIS* (ROSS) AND *ASCOGREGARINA TAIWANENSIS* (LIEN AND LEVINE) (APICOMPLEXA: LECUDINIDAE) FROM FLORIDA

Introduction

According to Christophers (1960), infections by *Ascogregarina culicis* in *Aedes aegypti* field populations from India were first reported by Ross in 1895. In the USA, infections of this gregarine in wild *Ae. aegypti* populations were first reported in 1968 (Barrett 1968). However, the description of *A. taiwanensis* as a new gregarine parasitizing *Ae. albopictus* in Taiwan is relatively recent (Lien and Levine 1980). In the USA, the presence of *A. taiwanensis* and field data about its prevalence in *Ae. albopictus* populations were observed originally in Illinois (Munstermann and Wesson 1990), which occurred a few years after the detection of breeding populations of *Ae. albopictus* in the USA (Sprenger and Wuithiranyagool 1986). Because both gregarines are host-specific (Beier and Craig 1985) it is likely that *A. taiwanensis* was introduced into the USA from Japan together with its host two decades ago (Munstermann and Wesson 1990), even though this gregarine has not been found in that country (Fukuda et al. 1997).

The pathogenicity of *Ascogregarina* spp. for their natural hosts is usually so low that they have been considered as "harmless" parasites (Beier and Craig 1985) or "commensals" in the mosquito midgut (Weiser 1963). However, some Asian (Sulaiman 1992) and American strains of *A. culicis* are pathogenic to *Ae. aegypti* (Barrett 1968). Recent reports have shown that *A. taiwanensis* did not cause any apparent mortality in North American *Ae. albopictus* (Garcia et al. 1994, Fukuda et al. 1997).

The injury that a gregarine is capable of causing to its host depends on the ability of sporozoites to pass through the peritrophic matrix and penetrate the larval midgut epithelial cells, and the efficiency of immunological mechanisms to encapsulate free trophozoites (Walsh and Olson 1976). In *A. culicis*, the sporozoites easily penetrate the larval stomach epithelial cells but many infected cells are rapidly eliminated through an apoptotic process (Weiser 1963) and immediately replaced by healthy cells. Furthermore, the interplay between the environment and the genetics of both parasite and host may be involved in the degree of detrimental effects. A result of the environment-genetic interaction is observed in the varying pathogenicity displayed by different geographical strains of *A. culicis* on its natural host *Ae. aegypti* (Sulaiman 1992).

Before 1985, *Ae. aegypti* was one of the most common and abundant mosquitoes in Florida, but ten years later, after the establishment of *Ae. albopictus*, the former has been replaced by the latter in most regions of Florida (O'Meara et al. 1995). At the present time, there is no clear explanation for this ecological phenomenon, which is a matter of controversy and has resulted in a number of hypotheses. One of these is the gregarine hypothesis, which is generally stated as "differential pathogenicity of gregarines in interspecies infections may mediate competitive interactions in nature" (Craig 1993). Apparently, *A. culicis* is a benign parasite for many strains of *Ae. aegypti* but it might have a detrimental impact upon the ability of *Ae. aegypti* adults to confront adverse environmental conditions (Hayes and Haverfield 1971). Likewise, there is a decrease in the wing-length and fecundity for *Ae. albopictus* females infected with *A. taiwanensis* (Comiskey et al. 1999). Thus far, only a few field surveys to estimate infection rates of *Ascogregarina* spp. in larval populations of both *Ae. aegypti* and *Ae. albopictus* have been conducted (Blackmore et al. 1995, Garcia et al. 1994). None of these studies on the host - parasite relationships of *Ascogregarina* - *Aedes* has evaluated the role of *A. culicis* and *A. taiwanensis* as factors in the competitive interactions between *Ae. aegypti* and *Ae. albopictus*. To address this issue, research must focus on the susceptibility of each host species to its own gregarine, cross-infection rates, and density-dependent pathogenicity (Blackmore et al. 1995) conducted with experimental populations collected from a locality where the competitive displacement process is in progress. The objectives of this laboratory study were to 1) determine the susceptibility of two strains of *Ae. aegypti* and *Ae. albopictus* to *A. culicis* and *A. taiwanensis*, and 2) to compare the pathogenicity of *A. culicis* and *A. taiwanensis* for their natural and alternate mosquito hosts.

Material and Methods

Wild larvae and pupae of *Aedes* spp. were collected from cemetery flowerpots in Tampa, FL 1999, returned to the laboratory and placed in a cage for emergence. Male and female adults of *Aedes aegypti* and *Ae. albopictus* were transferred to separate cages and provided a 10% sucrose solution on cotton pads over four days to allow for mating. After 4 days, adults were blooded on a guinea pig. A day after blood feeding, engorged females were individually placed into screen capped vials (50 ml) with approximately 10 ml distilled H_2O plus a small piece of paper for oviposition. After egg laying, adults were classified as healthy and infected by the presence of gametocytes with mature oocysts inside the lumen of Malpighian tubules, which were dissected from females in 5% Ringer's solution and observed with a phase-contrast microscope. Thus, a healthy and an infected colony of each *Aedes* species with its own gregarine were established to conduct the susceptibility bioassays of each host-gregarine system of the wild strains from Tampa. To examine differences in susceptibility that either *Ae. aegypti* or *Ae.*

albopictus have to their gregarines in nature, I established a laboratory strain of each mosquito species from colonies maintained at the Center for Medical, Agricultural and Veterinary Entomology (CEMAVE) from the USDA at Gainesville, FL. Wild (Tampa strain) as well as the insectary strain (USDA strain) of *Ae. aegypti* and *Ae. albopictus* were bioassayed by exposing them to oocysts of *Ascogregarina culicis* and *A. taiwanensis* respectively, which were originally isolated from the Tampa strain of both mosquito species.

Two experiments (one each for *Ae. aegypti* and *Ae. albopictus*) and two bioassays (the two strains of each mosquito species) per experiment were conducted. Each bioassay consisted of six treatments (five oocyst concentrations plus control), replicated three times. Oocysts used in these experiments were harvested from the infected colony of each *Aedes* species by macerating approximately 250 infected adult mosquitoes of each species with a tissue homogenizer in 5 ml distilled H_2O and then filtering through wet cotton in a glass syringe. The crude extract was centrifuged at 5,000 rpm for 10 minutes and oocyst concentrations determined with a haemocytometer and a phase-contrast microscope. A stock suspension of 10,000 oocysts/ml was prepared and oocyst concentrations of 6, 12, 25, 50 and 100 oocysts/larva, plus a control were established. Each treatment consisted of a group of 100 2^{nd}-instars placed in 100-ml distilled H_2O in a plastic cup (100 ml) with the oocysts and exposed for 24 hours. A small amount (0.04 g) of artificial larval diet (a 1:1 alfalfa: pig chow mix) was added to the test cups to promote oocyst ingestion and larval infection. After the 24-h exposure, the contents of each cup including water, oocysts and larvae were poured into an enamel pan containing 1 liter of distilled H_2O. Control cups were set up at the same time except that oocysts were not added to the water. Controls also were decanted to enamel pans, and both test and control pans were placed in an insectary at 27°C, 80% R. H., and a photoperiod of 14:10-h (D: L), where larvae were held throughout the remainder of their development. Three ml of a 5% larval diet (described above) were added to each pan at approximately 48 hours (2nd day) after hatching, and then 5 ml of the same suspension were added to each pan at approximately 120 hours (5th day) post-hatching.

To determine the number of gamonts in larvae, approximately 6 hours before pupation, 10 larvae were taken from each pan and midguts dissected in 5% Ringer's solution under a dissecting microscope at 16X magnification. The midgut of each larva was pulled from the body with sharp forceps after making two cuts: the first removed the head and the second at the middle of the last abdominal segment. Once the midgut was removed intact, accurate counts were made of all the gamonts present in the lumen between the epithelium and the peritrophic matrix of each specimen. The infection intensity was determined and expressed as gamont density/larva for the "Tampa strain" and "USDA strain" of *Ae. aegypti* exposed to the oocyst concentrations (treatments) of 6, 12, 25, 50 and 100 oocysts/larva of *A. culicis* plus the control in the 1st experiment. The same was done in the 2nd experiment for both strains of *Ae. albopictus* to *A. taiwanensis*. Dead larvae in all treatments were recorded. The pupation rate in all treatment groups was also recorded. The number of dead and live pupae per pan was counted and removed twice a day at about 10:00 AM and 2:00 PM over a period of approximately 6 days. Thus, pupae of each strain per species were recorded at approximately 12-h intervals to determine the pupation rate over time.

Statistical Analysis

The susceptibility of the two strains of each *Aedes* species to the infection of its corresponding *Ascogregarina* species was determined by a linear regression of gamont density/larva as the dependent variable upon the oocyst concentration/larva. The regression equation, Pearson correlation coefficient and ANOVAs were computed by using the SAS procedures PROC REG and PROC GLM, which included pair wise REGWQ tests. Means per treatment, strain, and replicate were calculated with the SAS procedure PROC MEANS, while PROC UNIVARIATE procedure was used to check data normalization. Mortality and pupation rates were analyzed with PROC PROBIT and PROC NPAR1WAY (SAS Institute 1988).

Results

Bioassays with *Aedes aegypti* Infected with *Ascogregarina culicis*, and *Ae. albopictus* Infected with *A.taiwanensis*

In general, the mean number of gamonts/larva varied significantly in function of the mosquito host, mosquito strain, and dose (oocyst concentration). In the analysis performed for each mosquito host, the mean number of *A. culicis* gamonts in *Ae. aegypti* larvae varied significantly depending on the strain and dose. In contrast the same analysis for the *A. taiwanensis* in *Ae. albopictus* revealed that the mean number of gamonts/larva did not vary by strain, only by dose (Table 2-1).

The *A. culicis* gamont intensity in the larval midgut of the *Ae. aegypti* “Tampa strain" had an overall average of 86.77 ± 9.33 gamonts/larva, which was significantly higher than the mean of 52.93 ± 9.41 gamonts/larva of the "USDA strain". In contrast, in the *Ae. albopictus* “Tampa strain” the *A. taiwanensis* gamont intensity averaged 52.83 ± 15.90 gamonts/larva, which was not significantly different from the 46.78 ± 8.52 gamonts/larva found for the “USDA strain” (Table 2-2). The higher intensity of infection for *A. culicis* in *Ae. aegypti* also was reflected in the larval infection rate. In *Ae. aegypti,* the average larval infection rate for the "Tampa strain” and “USDA strain” were 99 and

96%, whereas the infection rate of *A. taiwanensis* for both *Ae. albopictus* strains was 88 and 90%, respectively (Table 2-2).

As expected, the higher the oocyst concentration, the higher was the gamont intensity/larva in both host/parasite systems, and the correlation between both variables was high in both systems. However, the gamont intensity always tended to be higher in the "Tampa strain" of *Ae. aegypti* (Fig. 2-1 and 2-3).

The difference in susceptibility for each mosquito species infected with its own gregarine was more obvious in the linear regression of the gamont density/larva on oocyts concentration of the "Tampa strain" of both mosquito hosts. The increments in gamont intensity of *A. culicis* in function of the oocyst dose were higher in *Ae. aegypti* than those of *A. taiwanensis* in *Ae. albopictus*. The slope values in both linear regression equations were 1.98 and 1.49, respectively (Fig. 2-3).

Finally, the overall mortality rate expressed as the mean combined mortality rate (larvae + pupae) per replicate varied from 3.2 to 12.6% for all the oocyst concentrations tested within each strain per mosquito species. The mean mortality rates were not significantly different between host species and strains (Table 2-2).

Pupation Rate in *Ae. aegypti* Infected with *A. culicis*, and *Ae. albopictus* Infected with *A. taiwanensis*

To describe the pupation rate per species, the PT_{50} (Average number of hours in which 50% of pupation occurred per treatment, with the egg hatching as starting time) was estimated for each host/parasite combination by Probit analysis (% accumulated pupation against time in hours at 12-h intervals). However, to evaluate the impact by parasitism upon larval development time, I used the estimate PT_{50} as an indicator of larval development, whereby the PT_{50} was calculated only for two treatments: the highly infected (100 oocysts/larva) and the control. According to the chi-square ($\chi^2 = 5.41$, df =

4, p = 0.24) of a Kruskal-Wallis test, the PT_{50} values were not significantly different among the infected treatments and controls. Therefore, the larval development time was the same in both combinations *Ae. albopictus /A. taiwanensis* and *Ae. aegypti /A. culicis* (Table 2-3).

Discussion

This is the first study to determine with bioassays the susceptibility of two US strains of *Aedes aegypti* to *Ascogregarina culicis* and of *Ae. albopictus* to *A. taiwanensis.* The "USDA strain" and the wild "Tampa strain" of *Ae. albopictus* were equally susceptible to *A. taiwanensis*. The *Ae. albopictus* "USDA" colony was recently established with specimens collected from the Gainesville, Florida area when the "Asian tiger" was invading Florida in the second part of the 1980s. In contrast, in the *Ae. aegypti/ A. culicis* combination, the *Ae. aegypti* "Tampa strain" displayed a higher susceptibility to *A. culicis* than to the *Ae. aegypti* "USDA strain" which suggests that the two strains are genetically distinct. The USDA *Ae. aegypti* colony was established from the "Orlando strain" in the 1960s (Dr. J. J. Becnel, pers. comm.). In that long time, the host was not in contact with the gregarine. In addition, because of endogamy, maybe resistant genotypes to the infection of *A. culicis* appeared in the colony after 35 years of colonization.

This study was conducted with experimental populations of both host and parasite collected from a locality where *Ae. aegypti* is being displaced by *Ae. albopictus*. Prior to this report, there has been only one study in Asia (Thailand) by Sulaiman in 1992, in which bioassays were used to determine the susceptibility of *Ae. aegypti* to *A. culicis*. Sulaiman (1992) concluded that the gregarine pathogenicity depends on both strain and genetic compatibility between the gregarine and geographic strains of mosquito host.

Mortality observed in this study confirms the reports in the literature that the American strains of both *Ascogregarina* species are benign parasites in their natural hosts. Although the first report for the presence of *A. culicis* in the USA stated a significant mortality in *Ae. aegypti*, mortality data were not provided in the paper (Barrett 1968). In the case of *A. taiwanensis* larval mortalitity rates of up to 10% has been reported for both *Aedes* species (Garcia et al. 1994), which is in line with the larval-pupal mortality observed here ranging from 3.2 to 12.6% in both host/parasite systems.

My estimates of the mean infection intensities for the "Tampa strain"of *A. culicis* in *Ae. aegypti* (86.77 gamonts/larva) and *A. taiwanensis* in *Ae. albopictus* (52.83) are similar to those reported in the literature. A survey from twelve Florida cities to detect the prevalence of *A. culicis* in *Ae. aegypti* was conducted through the dry and wet seasons of 1968. The infection intensity varied from 1 to over 800 trophozoites/larva with an overall mean of 57 trophozoites/larva (Stapp and Casten 1971). In another Florida survey, the *Ascogregarina* spp. infection intensity ranged from 1 to 486 trophozoites/larva, and the average was higher in *Ae. aegypti* (52.5 gamonts/larva) than in *Ae. albopictus* (33.5 gamonts/larva) (Blackmore et al. 1995). It is interesting to note that in a survey done in Gainesville, FL, the average highest natural prevalence (January 1992) reported for *A. taiwanensis* in *Ae. albopictus* larvae collected from shaded tires was 64.7 ± 80.1 gamonts/larva (Garcia et al. 1994). Lastly, Sulaiman (1992) reported a gamont intensity of 68.9 gamonts/larva but with high mortality (>70%) for a dose of 100 oocysts/larva of the Korn Kean strain of *A. culicis*, which is the parasite strain normally found in the *Ae. aegypti* populations in the locality around Bangkok, Thailand. This infection intensity is almost the same that I found here (69.56 ± 2.42 gamonts/larva) when *Ae. aegypti* was infected with only 25 oocysts/larva and with only 3.9 % mortality.

When I infected larvae with 100 oocyst/larva the mean gamont intensity increased almost four times (214.80 ± 7.42 gamonts/larva) while the mortality was 7.3%, which means that the parasite sporozites from Florida were capable of invading and developing in the host epithelial cells at a rate four times higher that the rate observed in the *Ae. aegypti* Thailand strains.

The USA strains of *Ae. aegypti* and *Ae. albopictus* are able to tolerate high gamont intensities of *A. culicis* and *A. taiwanensis*, respectively, without significant mortality (Table 2-2). However, based on my results, I cannot conclude that these parasites are totally benign for their corresponding mosquito hosts, because they could impact host fitness. This was a preliminary study to gather information about basic host/parasite relationships. Further research involving cross and dual infections and more stressful conditions in mosquito larvae could demonstrate whether these gregarines play a role in the competition between both mosquito hosts and to favor one or another through the process.

Table 2-1. Sum of squares and F values from the ANOVA's conducted to determine the variation of mean gamonts/larva in six oocyst doses (0, 6, 12, 25, 50 and 100 oocysts/larva) of *Ascogregarina culicis* and *A. taiwanensisi* in two strains (USDA and Tampa) of *Aedes aegypti* and *Ae. albopictus.*

Source	Sum of Squares	Type III Sum of Squares	F	Pα
Model for both *Aedes* species	247,986.34		96.48	0.0001
Species		60,260.28	23.44	0.0001
Strain		59,660.48	23.21	0.0001
Dose		1; 367,997.29	133.05	0.0001
Model for *Ae. aegypti*	965,370.54		63.61	0.0001
Strain		85,885.92	50.94	0.0001
Dose		773,105.91	114.61	0.0001
Model for *Ae. albopictus*	607,005.15		40.08	0.0001
Strain		2,742.16	0.91	0.34 n.s.[1]
Dose		604,262.98	49.88	0.0001

[1] n. s =Not significant (p>0.05)

Table 2-2. Infection intensity expressed as mean gamonts/larva[1] in two strains of *Aedes aegypti* exposed to five oocyst concentrations of *Ascogregarina culicis*, and two strains of *Ae. albopictus* exposed to five oocyst concentrations of *A. taiwanensis*.

Species	Strain	Oocyst dose	Mean gamonts/ larva ± SE[2]	Infection %[3]	Mortality % [4]
Ae. aegypti	Tampa	6	18.87 ± 1.76	100	3.5
	Tampa	12	38.60 ± 5.73	100	4.0
	Tampa	25	69.56 ± 2.42	100	3.9
	Tampa	50	92.03 ± 18.33	97	7.1
	Tampa	100	214.80 ± 7.42	100	7.3
Average			86.77 ± 9.33**a**	99	5.16
	USDA	6	17.47 ± 1.58	87	5.3
	USDA	12	23.43 ± 3.61	93	6.4
	USDA	25	42.16 ± 11.91	100	3.2
	USDA	50	74.10 ± 19.65	100	4.7
	USDA	100	107.50 ± 13.75	100	8.3
Average			52.93 ± 9.41**b**	96	5.58
Ae. albopictus	Tampa	6	7.77 ± 0.76	70	4.2
	Tampa	12	15.17 ± 3.34	83	4.6
	Tampa	25	39.80 ± 15.29	90	4.9
	Tampa	50	51.03 ± 15.38	100	5.4
	Tampa	100	150.40 ± 32.84	97	5.3
Average			52.83 ± 15.90**a**	88	4.88
	USDA	6	10.00 ± 4.00	77	4.1
	USDA	12	17.27 ± 0.03	90	4.5
	USDA	25	35.43 ± 14.88	90	7.2
	USDA	50	53.33 ± 6.38	97	8.3
	USDA	100	117.90 ± 17.45	97	12.6
Average			46.78± 8.52**a**	90	7.34

[1] Mean gamonts /larva was calculated from n = 30 larvae.

[2] SE = Standard Error.

[3] Infection % = infected larvae in 30 dissected larvae. The average mean gamonts/larva per strain per species, with the same letter are not significantly different according to a REGWQ test (p>0.05) (SAS Institute 1988).

[4] Mortality = (average of dead larvae plus pupae/replicate = 90 larvae) x 100. The averages of mortality/replicate were not significantly different according to a Kruskal-Wallis test ($\chi 2 = 0.57$).

Table 2–3. Larval development (expressed as PT_{50}[1]) of *Aedes aegypti* infected with *Ascogregarina culicis* and *Ae. albopictus* infected with *A. taiwanensis*.

Host / parasite	Strain	PT_{50}[2]	95% Fiducial Limits (Lower, upper)
Ae. aegypti / A. culicis	Tampa	37.31	33.81, 40.72
Ae. aegypti / control	Tampa	31.38	27.19, 35.46
Ae. albopictus / A. taiwanensis	Tampa	45.00	40.42, 49.67
Ae. albopictus / control	Tampa	36.54	31.72, 41.38
Ae. aegypti / A. culicis	USDA	42.49	39.18, 45.73
Ae. aegypti / control	USDA	38.31	34.98, 41.55
Ae. albopictus / A. taiwanensis	USDA	47.80	43.66, 52.00
Ae. albopictus / control	USDA	41.83	38.21, 45.40

[1] PT_{50} = Average number of hours required for the 50% pupation in experimental larvae

[2] The PT_{50} values were not significantly different according to a Kruskal-Wallis test (χ^2 = 5.41, df = 4, p = 0.20).

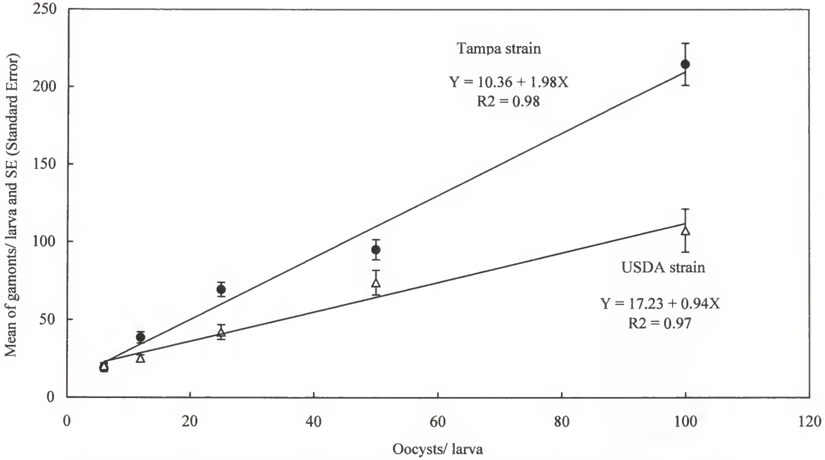

Fig. 2-1. Susceptibility of the Tampa (upper line) and USDA (lower line) strains of *Aedes aegypti* to *Ascogregarina culicis* exposed to five oocyst concentrations and expressed in gamont density/ larva ± SE (vertical line).

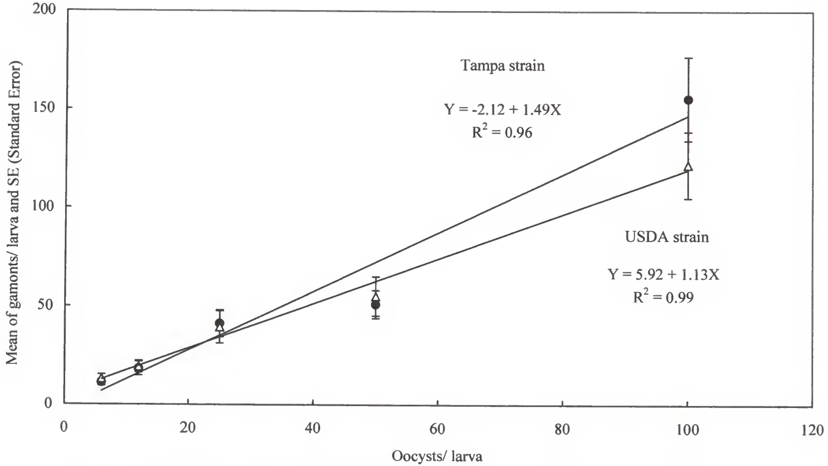

Fig. 2-2. Susceptibility of the Tampa (upper line) and USDA (lower line) strains of *Aedes albopictus* to *Ascogregarina taiwanensis* exposed to five oocyst concentrations and expressed in gamont density/ larva ± SE (vertical line).

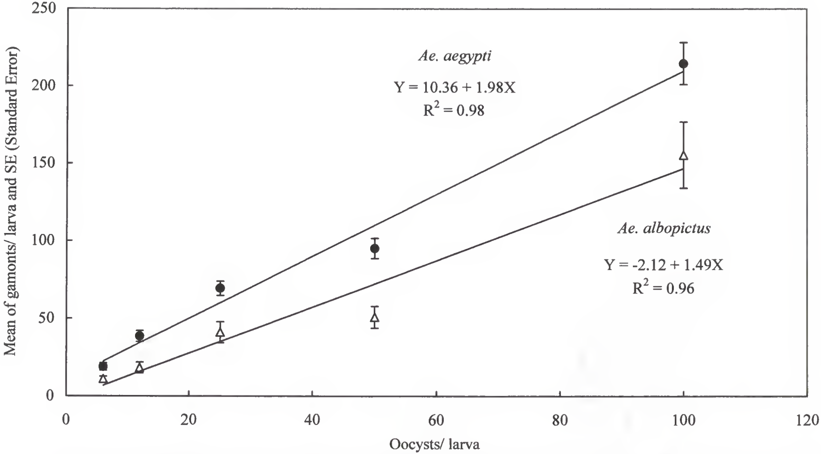

Fig. 2-3. Susceptibility of Tampa strains of *Aedes aegypti* (upper line) to *Ascogregarina culicis*, and *Ae. albopictus* (lower line) to *A. taiwanensis* exposed to five oocyst concentrations and expressed in gamont density/larva ± SE (vertical line).

CHAPTER 3

GAMONT DENSITY OF *ASCOGREGARINA CULICIS* AND *ASCOGREGARINA TAIWANENSIS* (APICOMPLEXA: LECUDINIDAE) IN LARVAE OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE), AND IMPACT OF INFECTION AND FOOD LEVELS ON HOST FITNESS

Introduction

The aseptate gregarines *Ascogregarina culicis* and *A. taiwanensis* are host-specific parasites of *Aedes aegypti* and *Ae. albopictus*, respectively (Beier and Craig 1985). These parasites are present at high prevalence rates in wild populations of their mosquito hosts in Central Florida (Blackmore et al. 1995).

The *Ascogregarina* life cycle occurs almost entirely within the mosquito host. Trophozoite growth and differentiation occurs in the larval midgut and gametocytes mature within the Malpighian tubules of pupae and adults. Within the gametocytes, gamete production takes place and fusion produces an oocyst containing eight sporozoites (Chen et al. 1996). Parasite dissemination depends on the ovipositional behavior of infected mosquitoes. Oocysts are expelled by females through the rectum during oviposition in larval breeding sites, or when either sex of infected adults defecates or dies, releasing oocysts, which are ingested by mosquito larvae (Beier and Craig 1985). Gregarine pathogenicity varies geographically. Some Asian strains of *A. culicis* are pathogenic to *Ae. aegypti* (Sulaiman 1992), while the USA strains of *A. culicis* (Barrett 1968) or *A. taiwanensis* (Fukuda et al. 1997) are considered non-pathogenic to their respective mosquito hosts. However, recent studies have demonstrated that *A.*

taiwanensis can exert a detrimental impact upon the fitness of *Ae. albopictus* adults (Comiskey et al. 1999).

Information concerning the host-parasite relationships for these gregarines is limited. Most studies have focused on determining *Ascogregarina* spp. infection rates in field populations of the mosquito hosts (Blackmore et al. 1995, Garcia et al. 1994) but only at the genus level because methods do not exist to distinguish the gamonts and/or oocysts of *A. culicis* from those of *A. taiwanensis* in dual-infected individuals collected in the field. Both *Aedes* species share the same larval ecological niche, and cross and dual infections occur in nature. The intensity and impact of these infections remains unknown. Because *Ae. albopictus* has been spreading throughout the United States and the distribution pattern of *Ae. aegypti* has diminished, I have conducted experiments to determine if *A. culicis* and *A. taiwanensis* affect the survival of sympatric populations of these two mosquito species. Specific objectives of this study were: 1) to evaluate the gamont intensity of each gregarine species when *Ae. aegypti* and *Ae. albopictus* larvae are exposed to one of the gregarine species or both species simultaneously, and 2) to measure the effect of a high oocyst concentrations (1000 oocysts/larva) and 2 food levels on the developmental time and size of *Ae. aegypti* and *Ae. albopictus* when exposed and reared sympatrically.

Material and Methods

Cultures of both gregarines were established *in vivo* as follows: Adults of *Ae. aegypti* and *Ae. albopictus*, from wild larvae and pupae collected in 1999 in flowerpots containing water in "El Asturiano" cemetery from Tampa, Florida, were separated and transferred to cages and held for four days to assure mating. They were fed on a 10%

sucrose solution provided on cotton pads and blood-fed on a guinea pig. A day after blood feeding engorged females were individually separated into screened-capped vials with approximately 10 ml distilled H_2O plus a small piece of filter paper for oviposition. After egg laying, adults were classified as healthy or infected by the presence of gametocytes with mature oocysts inside the lumen of Malpighian tubules, which were dissected from females in Ringer's solution and observed with a phase-contrast microscope. Thus, a healthy and infected colony of each *Aedes* species with its own gregarine was established.

A stock suspension of 1000 oocysts/ml of each gregarine sp. was prepared by macerating infected adult mosquitoes with a tissue homogenizer in distilled H_2O. The homogenate was filtered to eliminate host debris, and oocysts were counted on a hemocytometer. Groups of 100, 48-h-old healthy *Ae. aegypti* and *Ae. albopictus* larvae were exposed to the oocysts in 3.5 oz plastic cups in 100 ml of water plus 0.02% alfalfa and potbelly pig chow mixture (2:1). Groups without the addition of the gregarine served as controls. After an exposure of 24 h, the contents of each cup including water, oocysts and larvae were poured into an enamel pan containing 500 ml of distilled H_2O and placed into an insectary held at 27°C, 80% R. H., and 14:10-h (D: L). The larval diet was a 5% alfalfa and potbelly pig chow mixture (2:1) of which three ml were added to each pan at 48 hours post-exposure and then 5 ml of the same suspension were added to each pan at 120 hours post-exposure.

Larval guts were dissected in Ringer's solution and placed under a glass cover slip to count gamonts with a phase-contrast microscope. To determine gamont numbers, midguts of larvae were removed from the body with sharp forceps after making two cuts, the first at the anterior thorax and the second at the middle of the last abdominal segment. Once the midgut was removed intact, I was able to do accurate counts of all the gamonts present in the lumen between the epithelium and the peritrophic membrane of each specimen.

Four experiments were performed. In the first experiment, the density of gamonts/larva for each gregarine per mosquito host species was determined in mixed larval treatments (50 *Ae. aegypti* + 50 *Ae. albopictus* larvae) that were singly and dually infected with *A. culicis* and/or A. *taiwanensis.* The second and third experiment determined the single and cross-infection rates of both gregarines (100 oocysts/larva) in *Ae. aegypti* and *Ae. albopictus* larvae. Lastly, the fourth experiment consisted of mixed larval treatments (50 *Ae. aegypti* + 50 *Ae. albopictus* larvae), in which larval development time and adult size were determined from larvae exposed to both gregarines and reared at high and low food levels. Each treatment was twice replicated. A control (0 oocysts/larva) and a high (1000 oocysts/larva) infection level were used. Larvae were fed at 2 food levels, standard (5 g/100 ml distilled H_2O) and low (1.25 g/100 ml distilled H_2O). For each level, 5 ml of the diet (described in Chapter 2) were added to each pan at 48 hours (2nd day) and 120 hours (5th day).

To determine larval development time (LDT), pupation was recorded twice each day (10:00 AM and 8:00 PM) and pupae were individually separated into screened-capped plastic vials with approximately 10 ml distilled H_2O. Each vial was labeled with the pupation date and treatment, and two raisins were placed on the screen as a carbohydrate source for adults. All adults were individually separated and anesthetized with CO_2, and the left wing was dissected and measured with a 3.2X micrometer. Wing-length was measured from the axial junction to the distal margin excluding the fringe (Washburn et al. 1989). Infection for each treatment was assessed by squashing individually the body of 5 males and 5 females, that were examined with phase-contrast microscopy to confirm the presence of oocysts.

Statistical Analysis

Mean gamont density/larva was used as a measure to evaluate the intensity of single, dual and cross-infections in single and mixed larval treatments. Mean number of gamonts/larva among the treatments was analyzed by ANOVAs with the procedures PROC GLM, PROC UNIVARIATE, and PROC MEANS. The same procedures plus the PROC TTEST were applied for the analysis of mean wing-length in millimeters and mean LDT in days for the adults that emerged from the larval treatments. Original data from both variables were transformed. The logarithmic function (log $(X + 1)$) was used for wing-length, while the square root ($\sqrt{X+1}$) for LDT. Food levels, mosquito species, sex and infection were the independent variables. All statistical procedures were conducted with SAS (SAS Institute 1988).

Results

Differentiating *Ascogregarina culicis* and *A. taiwanensis* Gamonts in Dual-Infected Larvae of *Aedes aegypti* and *Ae. albopictus*, by using Morphological Features

After multiple larval dissections of each mosquito species with single and dual infections, I detected differences in the shape and pigmentation between the gamonts of *A. culicis* and *A. taiwanensis* at six days post infection (approximately 5 h before pupation) when viewed with phase contrast microscopy. Gamonts of *A. culicis* were always translucent either inside (Fig. 3-2 and 3-3) or outside the gut (Fig. 3-1). When dissected guts were placed extended on the slides, the *A. culicis* gamonts always had a "big-head" appearance because the gamonts' anterior part comprising the protomerite and epimerite was perceptibly wider than the tapered deutomerite (Fig. 3-3). In contrast, the *A. taiwanensis* gamonts had a short and untapered deutomerite and were primarily distinguished by brown pigmentation when they were observed either inside (Fig. 3-2 and 3-4) or outside the digestive tract (Fig. 3-1). When both gamont species were outside the gut, they were elongated but *A. culicis* gamonts were longer than those of *A taiwanensis* (Fig. 3-1). Therefore, gamonts of the two species could be distinguished by pigmentation and shape. The *A. taiwanensis* gamonts were always brown and bulbous, whereas no color was observed in the *A. culicis* gamonts in larvae. In pupae, however, there was no difference in shape but the difference in pigmentation between the two species remained.

Gamont Density Results of *Ascogregarina culicis* and *A. taiwanensis* in Single, Dual and Cross-infections in *Aedes aegypti* Larvae

The mean gamont intensity/larva for *A. culicis* and *A. taiwanensis* varied significantly in larval treatments that consisted of one or both *Aedes* species in single and dual exposures (Table 3-1). Although *A. culicis* and *A. taiwanensis* can infect an alternate host, there was a clear specificity for the natural hosts (Table 3-3). Although not significant, *A. taiwanensis* tended to more consistently establish infections in *Ae. aegypti* than *A. culicis* in *Ae. albopictus*. The highest infection intensities of *A. taiwanensis* were found in single (5.05 gamonts/larva) and dual (2.0 gamonts/larva) exposures made with pure populations of *Ae. aegypti*. In contrast, *A. culicis* was able to establish infections only in pure populations of *Ae. albopictus* exposed to both gregarines (1.00 gamonts/larva).

The presence of *A. taiwanensis* significantly inhibited the infection intensity of *A. culicis* in *Ae. aegypti* when dual exposures were made in either single or mixed larval populations. As expected, infection intensities of *A. culicis* were greatest (202.45 gamonts/larva) in single exposures made with pure populations of *Ae. aegypti*, but the presence of *A. taiwanensis* significantly reduced the *A. culicis* intensities to 103.85 gamonts/larva (Table 3-3). Likewise, in exposures made with mixed mosquito populations, gamont intensities in *Ae. aegypti* were highest with single exposures of *A. culicis* (143.20) and were significantly reduced (68.85) when *A. taiwanensis* was present (Table 3-3). In contrast, the infection intensities of *A. taiwanensis* in *Ae. albopictus* were not changed (87.40) in single exposures made in pure populations of *Ae. albopictus* and was not different (62.00) when *A. culicis* was present (Table 3-3). The lowest infection intensities of *A. taiwanensis* were observed with mixed larval populations in either single (46.35) or dual exposures (48.25).

A 4th experiment was conducted to evaluate the effect of food on adult size and larval development in mixed populations of *Ae. aegypti* and *Ae. albopictus* exposed to 2 concentrations of each gregarine (infected and control treatments). Adult body size varied as a function of food, species and sex, but not infection, while larval development time varied as a function food, infection but not of mosquito species (Table 3-1). To examine in detail the effects of parasitism, the variation of body size and development time was analyzed within each sex for each mosquito species by food level. Adult body size and development time varied significantly in both mosquito host and food level (Table 3-2).

Wing-Length

Although the body size of both *Ae. aegypti* and *Ae. albopictus* was not affected by the parasitism (Table 3-1), the analysis of the mean wing-length within the same species for each food level was conducted as an attempt to see differences in size not detected in the general analysis. Then, the analyses carried out for each mosquito species demonstrated that a reduction in body size by parasitism was clear only in *Ae. aegypti* males that emerged from larvae in the low food treatments. Males that emerged from heavily infected larvae were smaller size (1.93 ± 0.03 mm) than those from the uninfected larvae (2.01 ± 0.04 mm), while in the rest of treatments the size within groups did not vary significantly (Table 3-4).

Larval Development Time

Because the general analysis demonstrated that larval development time varied as a function of food level, sex and parasitism, but not between mosquito hosts (Table 3-1), I conducted additional analyses to detect variation in means within the same sex per species as I did for body size. In the high food test, development time of parasitized *Ae. aegypti* males was significantly extended (6.82 ± 0.09 d), while *Ae. albopictus* was unaffected by parasitism. In the low-food treatments, parasitism prolonged the development time of *Ae. aegypti* females (11.60 ± 0.57 d) but not in males (Table 3-5). In contrast, in *Ae. albopictus,* only the males presented a significant longer development (9.04 ± 0.33 d) than those uninfected males (8.77 ± 0.16 d) (Table 3-5).

Discussion

Differentiating of the *A. taiwanensis* Gamonts from Those of *A. culicis* in Dual-Infected Larvae

While I believe that both *Ascogregarina taiwanensis* and *Aedes albopictus* are the same species described by Lien and Levine (1980) from Taiwan, it is possible that I have a new isolate of *A. taiwanensis* in *Ae. albopictus* from Florida. Lien and Levine (1980) did not report differences in pigmentation between *A. culicis* and *A. taiwanensis* in their detailed descriptions and specifically stated that they were hesitant to try and separate these two species based on the structure of their gamonts and gametocytes. In addition, the morphology of the gamonts of *A. taiwanensis* reported here appears to be distinctive from those illustrated by Munsterman and Wesson (1990) and Garcia et al. (1994). The morphological differences reported here may indicate that this is a new geographic isolate of *A. taiwanensis* from Florida.

Because the phase contrast microscope is a common tool used by insect pathologists, it is surprising that a simple morphological difference in pigmentation color in gamonts between both gregarines had not been noted previously (Blackmore et al. 1995, Garcia et al. 1994, Fukuda et al. 1997). Perhaps, this is because in the past studies the authors did not conduct numerous cross-infections in both mosquito larvae by infecting them with oocysts extracted from wild mosquito species. This was the only way in which it was recognized that there was a difference in pigmentation between *A. culicis* and *A. taiwanensis* gamonts after conducting many dissections of single and dual-infected larvae.

Additionally, I made many preliminary observations before starting this study. I dissected many *Ae. albopictus* larvae hatched from eggs from the USDA colony that I infected with oocysts extracted from wild *Ae. albopictus* adults collected in Gainesville, FL, where the mosquito is abundant in the CEMAVE (USDA) compound during the summer and fall of 1998. I took many pictures of the gamonts we found in those larvae, and the cytoplasmic pigmentation was always brown-colored, as I found in *A. taiwanensis* gamonts, in the experiments of this study.

Unfortunately, I did not take measurements such as gamont length or width: length ratio, but it is well known that size in *A. taiwanensis* gamonts is quite variable.

Mature gamont size in both *Ascogregarina* species is extremely variable and depends largely on the host. *A. culicis* gamonts in *Ae. aegypti* have a length of 275-280 μm and a width of 19-20 μm , while gamont size for *A. taiwanensis* is quite variable from different geographic isolates with a mean for a strain of *Ae. albopictus* from Florida of 53.3 ± 7.1 x 26.5 ± 1.9 μm (Garcia et al. 1994), a size much smaller than 234 x 32 μm which was originally reported for an isolate from Taiwan by Lien and Levine 1980. Many factors might affect the gamont size and shape such as host species, larval food, plus the possible cell distortion exerted by the pressure of the cover slip on the slide during the observations of gamonts with the dissecting microscope.

Gamont Density of each *Ascogregarina* Species in Both *Aedes* Species Mixed Larvae

Under comparable dose and exposure conditions, the gamont densities of *A. culicis* in *Ae. aegypti* were generally two to three times greater than the densities of *A. taiwanensis* found in *Ae. albopictus*. The reason for this reduction is not known but insects are reported to resist midgut infections by desquamation (excretion) of midgut cells into the lumen of the digestive tract (Tanada and Kaya 1993). Surprisingly, the gamont densities of *A. culicis* in *Ae. aegypti* were reduced by approximately 50% when *A. taiwanensis* was present at the time of exposure. Because *A. taiwanensis* only rarely became established in *Ae. aegypti*, the lower densities of *A. culicis* may result from fewer attachment points in the midgut caused by the loss of cells to which *A. taiwanensis* attached.

Although it has been reported that *A. taiwanensis* is unable to complete its life cycle in hosts other than *Ae. albopictus* (Lien and Levine 1980), *Ascogregarina* species are not host-specific parasites in mosquitoes. Recently, oocysts recovered from *Ae.*

taeniorhyncus adults that were infected with oocysts extracted from *Ae. albopictus*, (presumably *A. taiwanensis*) were infectious for *Ae. aegypti*, *Ae. albopictus* and *Ae. taeniorhyncus* larvae. (Garcia et al. 1994). This capability of *A. taiwanensis* to complete its life cycle in alternate hosts may represent an adaptive strategy for long-term survival. Many basic aspects on the biology of both *A. taiwanensis* and *A. culicis* remains unknown. An early study demonstrated that *A. taiwanensis* was able to infect *Ae. aegypti* larvae at a rate of 48% (Lien and Levine 1980). In a more recent study, infection rates of 56.3 and 12% in *Ae. aegypti* larvae by *A. taiwanensis* were induced in the laboratory and included the recovery of infective oocysts from *Ae. aegypti* adults (Munstermann and Wesson 1990).

Wing-Length

Adult mosquito body size is a physical expression of the nutritional value of the habitat (Nasci and Mitchell 1994) and for both *Aedes* species studied here, the wing-length is a measure significantly correlated with body size. Nasci (1986) reported a highly significant correlation between wing-length and fecundity ($r^2 = 0.92$ and 0.59) for *Ae. aegypti* and *Ae. albopictus* colonies, respectively.

There were no differences in body size of mosquitoes emerged from the high food, as a result of exposure to the gregarines (Table 3 -4). However, in the low food treatment group, there was a reduction in size exerted by parasitism on *Ae. aegypti* males of approximately 5% in comparison with the controls. This negative impact on mosquito males by gregarine parasitism was reported in previous studies. There was a similar size reduction (6%) in *Ae. albopictus* males reared under a low diet regime and infected with 2,000 oocyst/larva of *A. taiwanensis* (Comiskey et al. 1999). However, in *Ae. triseriatus* infected by *A. barretti* at a tire dump in Illinois, a stronger size reduction occurred in females (8-30%) than in males (3-10%) (Siegel et al. 1992). In another study of *Ae. triseriatus* larvae infected with 2000 oocysts/larvae by *A. barretti* there was a decrease of approximately 9% in female pupa weight, while no effect was found for male pupae (Beier 1980).

Larval Development Time.

It terms of development time I found that *Ae. aegypti* was more susceptible to A. culicis than *Ae. albopictus* to *A. taiwanensis*. The prolonged development time was expressed in males and the effect was observed in the well fed larvae. In the low-food treatment, the females of *Ae. aegypti* also exhibited a larval prolonged development in the infected treatments, while in *Ae. albopictus* this effect was observed only in males.

Therefore, the gregarines influence the fitness of both *Aedes* species in terms of body size and larval development time, whereby the Craig's hypothesis is true in relation to the negative impact that these gregarines are capable to do on their hosts. However, further research in field must be conducted to demonstrate whether this influence also is present in larvae of both mosquito hosts infected with their corresponding gregarines and living simpatrically in tires.

Table 3-1. Type III Sum of squares (SS)[1] and F values (Experiment 4) for food, species, sex and infection factors as variation sources in a two-way ANOVA for the dependent variables wing-length (log X + 1) and larval development time in days ($\sqrt{X+1}$)of *Aedes aegypti* and *Ae. albopictus*, mixed and infected by *Ascogregarina culicis* and *A. taiwanensis*.

Variable	Source	df	SS	F	Pα level
Wing-length	Model	4	1.106	556.82	0.0001
	Food	1	0.322	649.92	0.0001
	Species	1	0.054	110.73	0.0001
	Infection	1	0.000026	0.05	0.81 n.s.[2]
Development	Model	4	50.00	162.57	0.0001
	Food	1	34.08	443.20	0.0001
	Species	1	0.243	3.16	0.07 n.s.[2]
	Infection	1	0.767	9.98	0.0017

[1] Excepting the Sum of Squares for the model.

[2] n.s. Not significant (p>0.05)

Table 3-2. Values of t and significance level (P> | t |) for wing-length and larval development time[1] for adults infected and not infected within the same sex, of *Aedes aegypti* and *Ae. albopictus* emerged from a high and a low food levels, and from larvae exposed to 1000 oocysts/larva of *Ascogregarina culicis* and *A. taiwanensis*, in mixed larval treatments with dual infections.

Food Level	Dependent Variable	Species	Sex[2]	No. of Emerging Adults[3]	t*	df	P> | t |
High	Wing-Length	*Ae. aegypti*	F	54	-1.73	52	0.0881
			M	80	-1.38	78	0.1705
		Ae. albopictus	F	46	-1.97*	44	0.0557
			M	56	-1.13	54	0.2608
Low		*Ae. aegypti*	F	66	0.13	64	0.8932
			M	107	2.07*	105	0.0400*
		Ae. albopictus	F	28	-1.18	26	0.2460
			M	51	0.15	49	0.8739
High	Development	*Ae. aegypti*	F	54	0.76	52	0.4491
			M	80	-2.35*	78	0.0211
		Ae. albopictus	F	46	-2.59*	44	0.0129
			M	56	-1.07	54	0.2868
Low		*Ae. aegypti*	F	66	-2.72*	64	0.0142*
			M	107	-1.76	105	0.0809
		Ae. albopictus	F	28	-0.39	26	0.6967
			M	51	-0.72	49	0.4696

[1] Wing-length and development time were recorded in the same individuals as adults and larvae

[2] F = female, M = male.

[3] No of adults emerged from 200 larvae of each *Aedes* species.

* t values significant at $p < 0.05$.

Table 3-3. Mean gamont density ± Standard Error (SE) of *Ascogregarina culicis* and *A. taiwanensis* in 4th instar larvae of *Aedes aegypti* and *Ae. albopictus* single or dual-infected as 24-h old larvae, during 24-h with a dose of 100 oocysts/larva of each gregarine.

Mosquito host	Exposure	Parasite	Mean ± SE[4]
Ae. aegypti[1]	Single	*A. culicis*	143.20 ±14.61 **a**
Ae. aegypti	Single	*A. taiwanensis*	0.85 ± 0.31 **b**
Ae. aegypti	Dual	*A. culicis*	68.85 ± 10.05 **c**
Ae. aegypti	Dual	*A. taiwanensis*	0.15 ± 0.08 **b**
Ae. albopictus	Single	*A. culicis*	0 **a**
Ae. albopictus	Single	*A. taiwanensis*	46.35 ± 12.80 **b**
Ae. albopictus	Dual	*A. culicis*	0± **a**
Ae. albopictus	Dual	*A. taiwanensis*	48.25±12.16 **b**
A. aegypti[2]	Single	*A. culicis*	202.45±14.60 **a**
A. aegypti	Single	*A. taiwanensis*	5.05 ± 0.95 **b**
A. aegypti	Dual	*A. culicis*	103.85± 12.94 **c**
A. aegypti	Dual	*A. taiwanensis*	2.00 ± 0.78 **b**
Ae. albopictus[3]	Single	*A. culicis*	0 **a**
Ae. albopictus	Single	*A. taiwanensis*	87.40 ± 15.42 **b**
Ae. albopictus	Dual	*A. culicis*	1.00 ± 1.00 **a**
Ae. albopictus	Dual	*A. taiwanensis*	62.00 ± 13.23 **b**

[1] Experiment 1. Mixed larvae single and dual infected.

[2] Experiment 2. Only *Ae. aegypti* exposed to single and dual infections.

[3] Experiment 3. Only *Ae. albopictus* exposed to single and dual infections.

[4] Means with the same letter are not significantly different according to a REGWQ test (p>0.05) (SAS Institute 1988).

N = 20 larvae

Table 3-4. Wing-length (mm) in *Aedes aegypti* and *Ae. albopictus* females and males from mixed larvae reared under two food levels and infected[1] with 1000 oocysts/larva of each *Ascogregarina culicis* and *A. taiwanensis* in dual exposures.

Food	Species	Sex	Infection	N[2]	Mean ± Standard Error[3]
Standard	*Ae. aegypti*	Female	0	31	2.90 ± 0.01 **a**
	Ae. aegypti	Female	1000	24	2.95 ± 0.01 **a**
	Ae. aegypti	Male	0	45	2.26 ± 0.01 **a**
	Ae. aegypti	Male	1000	35	2.28 ± 0.01 **a**
	Ae. albopictus	Female	0	33	2.68 ± 0.02 **a**
	Ae. albopictus	Female	1000	13	2.76 ± 0.03 **a**
	Ae. albopictus	Male	0	26	2.17 ± 0.01 **a**
	Ae. albopictus	Male	1000	30	2.20 ± 0.01 **a**
Low	*Ae. aegypti*	Female	0	28	2.31 ± 0.04 **a**
	Ae. aegypti	Female	1000	23	2.44 ± 0.05 **a**
	Ae. aegypti	Male	0	17	2.01 ± 0.04 **a**
	Ae. aegypti	Male	1000	21	1.93 ± 0.03 **b**
	Ae. albopictus	Female	0	12	2.22 ± 0.05 **a**
	Ae. albopictus	Female	1000	16	2.41 ± 0.08 **a**
	Ae. albopictus	Male	0	24	1.97 ± 0.05 **a**
	Ae. albopictus	Male	1000	22	1.88 ± 0.02 **a**

[1] The infection = 0 is the control

[2] N= number of emerged adults from 200 larvae.

[3] Means with the same letter within the same species are not significantly different according to a Student t test for unpaired means ($p > 0.05$) (SAS Institute 1988).

Table 3-5. Larval development time in days ($\sqrt{X+1}$) in *Aedes aegypti* and *Ae. albopictus* females and males from mixed larvae reared under two food levels and infected with 1000 oocysts/larva of each *Ascogregarina culicis* and *A. taiwanensis* in dual exposures.

Food	Species	Sex	Oocyst Concentration[1]	N (Adults)	Mean ± Standard Error[2]
Standard	*Ae. aegypti*	Female	0	30	7.33 ± 0.12 **a**
	Ae. aegypti	Female	1000	24	7.20 ± 0.10 **a**
	Ae. aegypti	Male	0	45	6.53 ± 0.08 **a**
	Ae. aegypti	Male	1000	35	6.82 ± 0.09 **b**
	Ae. albopictus	Female	0	33	7.45 ± 0.10 **a**
	Ae. albopictus	Female	1000	13	8.15 ± 0.33 **a**
	Ae. albopictus	Male	0	26	6.34 ± 0.09 **a**
	Ae. albopictus	Male	1000	30	6.50 ± 0.10 **a**
Low	*Ae. aegypti*	Female	0	28	9.71 ± 0.38 **a**
	Ae. aegypti	Female	1000	38	11.60 ± 0.57 **b**
	Ae. aegypti	Male	0	62	8.64 ± 0.18 **a**
	Ae. aegypti	Male	1000	45	9.20 ± 0.26 **a**
	Ae. albopictus	Female	0	12	11.50 ± 0.45 **a**
	Ae. albopictus	Female	1000	16	12.00 ± 1.04 **a**
	Ae. albopictus	Male	0	27	8.77 ± 0.16 **a**
	Ae. albopictus	Male	1000	24	9.04 ± 0.33 **b**

[1] Treatment = 0 is the control

[2] Means with the same letter within the same sex per species are not significantly different according to a Student t test (PROC TTEST) (p >0.05) (SAS Institute 1988).

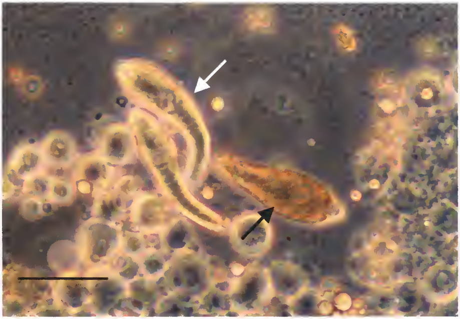

Fig. 3-1. Gamonts of *Ascogregarina culicis* (white arrow) and *Ascogregarina taiwanensis* (black arrow) outside the midgut of *Aedes aegypti* larvae infected with 100 oocysts/larva of each gregarine (fresh, phase-contrast). Bar, 50 µm.

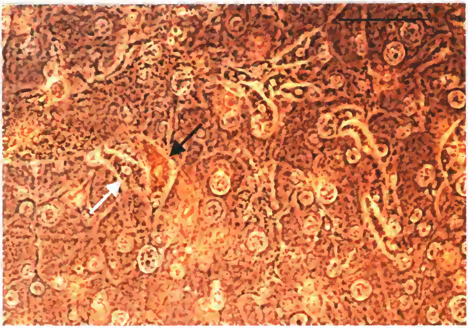

Fig. 3-2. Gamonts of *Ascogregarina culicis* (white arrow) and *Ascogregarina taiwanensis* (black arrow) inside the midgut of *Aedes aegypti* larvae infected with 100 oocysts/larva of each gregarine (fresh, phase-contrast). Bar, 100 µm.

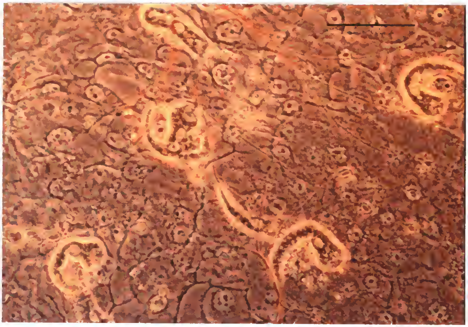

Fig. 3-3. Gamonts of *Ascogregarina culicis* within the midgut of *Aedes aegypti* larvae infected with 100 oocysts/larva of each gregarine (fresh, phase-contrast). Bar, 100 μm.

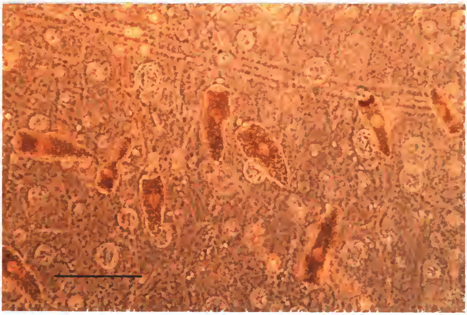

Fig. 3-4. Gamonts of *Ascogregarina taiwanensis* within the midgut of *Aedes aegypti* larvae infected with 100 oocysts/larva of each gregarine (fresh, phase-contrast). Bar, 100 μm.

CHAPTER 4

EFFECTS OF PARASITISM BY *ASCOGREGARINA TAIWANENSIS* (LIEN AND LEVINE) AND *ASCOGREGARINA CULICIS* (ROSS) ON LARVAL COMPETITION BETWEEN *AEDES ALBOPICTUS* (SKUSE) AND *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE)

Introduction

Competition is an interaction between individuals of the same or different species that share a resource, which is in limited supply. Consequently, a reduction in survivorship, growth and reproduction of the competing species may occur (Begon et al. 1990). Because other factors such as predation can maintain populations below levels in which competition occurs, it is very difficult to determine the extent that competition is affecting natural populations. In competition, the species involved potentially are always negatively impacted, and one strategy commonly exhibited by competitors is to avoid competition wherever possible (Speigh et al. 1999).

Sometimes parasites alter the expected result of competition. A classic study of competition altered by parasitism was published by Park (1948), who analyzed competition between *Tribolium confusum* and *T. castaneum* when each was infected with the coccidian *Adelina tribolii.* He observed that *T. castaneum* becomes extinct when the parasite is present, and *T. confusum* becomes extinct when *A. tribolii* is absent.

An analogous situation could prevail in the competitive interaction occurring in wild populations of the mosquitoes *Ae. aegypti* and *Ae. albopictus* where larvae of both species share the same breeding sites, such as flower pots or discarded tires. Laboratory experiments on larval competition between North American strains of both *Ae. albopictus* and *Ae. aegypti* under limited and optimal food conditions have demonstrated that the former is an inferior competitor to the latter. Interspecific competition slightly increased the development time of *Ae. albopictus*, but no effect was observed on its survival. By contrast, *Ae. aegypti* exhibited a higher survival and fecundity than *Ae. albopictus*, while body size remains equal in both species (Black et al. 1989). However, recent field studies conducted by Juliano (1998) with mixed species populations in tires, showed that *Ae. albopictus* is a superior competitor under larval conditions of stress and displays a higher survivorship to adulthood than that of *Ae. aegypti*. He found that the outcome depended on competition intensity and concluded that *Ae. aegypti* predominated only where interspecific competition with *Ae. albopictus* was not intense. Although he did not identify the *Ascogregarina* species, Juliano (1998) observed moderate parasitism (21.8%) levels in *Ae. albopictus* larvae and low rates (0.8%) of parasitism in *Ae. aegypti* larvae in experimental tires, and concluded that *Ascogregarina taiwanensis* did not play an important role in competition between these mosquito species in the field.

Some wild populations of both mosquito species exhibit high prevalence rates of the host-specific gregarines *A. culicis* and *A. taiwanensis* (Beier and Craig 1985). Following the introduction of *Ae. albopictus* in the USA in 1986 (Sprenger and Wuithiranyagool 1986), a decline in *Ae. aegypti* abundance and distribution has been observed through the eastern part of the country (Moore 1999). Its complete disappearance in northcentral Florida has now occurred (O'Meara et al. 1995). *A. culicis* prevalence in *Ae. aegypti* populations may be 95% in field collections with an infection intensity varying from 150 to 800 trophozoites/larva (Walsh and Olson 1976). By contrast, the infection intensity of *A. taiwanensis* in *Ae. albopictus* tends to be lower, with an average of 23.4 gamonts/larva and a range of 1 to ~300 trophozoites/larva (Garcia et al. 1994). According to a recent field study conducted in Florida where the gregarine species were not identified, *Ae. aegypti* showed a higher parasite load than *Ae. albopictus* with a mean of 52.5 and 33.5 gamonts/larva, respectively (Blackmore et al. 1995). Infection by these gregarines does not cause a significant mortality in their hosts, but parasites may decrease adult fitness in both mosquito species (Barrett 1968, Comiskey et al. 1999).

Craig (1993) proposed that competition between these *Aedes* species may be mediated by their gregarines. *Ascogregarina culicis* and *A. taiwanensis* have been considered as benign parasites of their natural hosts *Ae. aegypti* and *Ae. albopictus*, respectively (Stepp and Casten 1975, Garcia et al. 1994). However, there is no information to determine whether these parasites exert a negative impact on fitness of their respective hosts when they are under competition.

The present study was designed to explore and describe the populations consequences when larvae of these two closely related species of mosquitoes, *Ae. aegypti* and *Ae. albopictus*, are infected with their host-specific gregarines *A. culicis* and *A. taiwanensis*, and then placed into tires under field conditions where larval competition would be expected.

Material and Methods

Gregarine Culture and Mosquito Colony Establishment

Immature stages of *Ae. aegypti* infected with *A. culicis*, and *Ae. albopictus* infected with *A. taiwanensis* were collected in “El Asturiano” cemetery located in Tampa, FL in 1999 where the two species co-exist (O’Meara , pers. comm.). Emerging adults were used to colonize both mosquito species in the Biological Control Laboratory of the

CEMAVE (Center for Medical, Agricultural, and Veterinary Entomology) in the USDA-ARS at Gainesville, FL. Adults of each species emerging from wild larvae and pupae were transferred to separate cages and held for four days for mating. They were fed on a 10% sucrose solution provided in cotton pads and blood-fed on a guinea pig. A day after blood feeding, engorged females were individually separated into screened-cap vials with approximately 10 ml distilled H_2O. After oviposition, adults were classified as healthy or infected by the presence of gametocysts with mature oocysts inside the lumen of Malpighian tubules that were dissected from females in Ringer's solution and observed with a phase-contrast microscope.

To maintain infected colonies, approximately 100 adults were removed from the colony of each mosquito species, macerated, filtered through cotton in a glass syringe, and the crude extract was centrifuged at 5,000 rpm for 10 minutes. A stock suspension of 10,000 oocysts/ml was prepared and used to infect 24-h-old larvae confined in a plastic cup containing 100 ml distilled H_2O with 100 oocysts/larva. Larvae were exposed to the oocysts for 24 hours with 0.1 g of food per cup (1:1 Hogchow: alfalfa) to enhance the infection of larvae (J. J. Becnel, pers. comm.). They were held at 28 ^{o}C, 80% RH, and a photoperiod 10:14 (L: D) h.

Oocyst Extraction, Infection and Experimental Unit Establishment

The experiment was conducted at the University of Florida (FMEL) at Vero Beach in June – July 2000. Gregarine oocysts to infect the larvae, as well as eggs of both mosquito species to set up the experimental units (1 unit = 100 larvae), were from the F6 generation from the colonies held at the CEMAVE (USDA-ARS) at Gainesville FL. For both species, egg hatch was carried out on the same day. For each species, 3 small pieces of paper with attached eggs were placed into a 100-ml plastic cup filled with distilled

H_2O. Brewer's yeast (0.10 g) was added to each cup and approximately 5000 eggs contained in 8 100-ml plastic cups with water were set into a glass chamber vacuum for hatching. Twenty-four h after hatching, larvae were counted in enamel pans with a dropper and 30 groups of 100 larvae/cup were established.

Experimental treatments consisted of 1) controls of 100 healthy *Ae. aegypti* larvae (AEG H ALONE), 2) controls of 100 healthy *Ae. albopictus* larvae (ALBO H ALONE), 3) a mixed control of 50 healthy larvae of each species (BOTH H), 4) a mixed infection of 50 *Ae. aegypti* larvae infected with 1000 oocysts/larva of *Ascogregarina culicis* plus 50 non-infected *Ae. albopictus* larvae (AEG INF / ALBO H), 5) a mixed infection of non-infected *Ae. aegypti* larvae plus 50 *Ae. albopictus* larvae infected with 1000 oocysts/larva of *A. taiwanensis* (AEG H / ALBO INF); 6) a dual infection of 50 *A. culicis*-infected *Ae. aegypti* larvae plus 50 *A. taiwanensis*-infected *Ae. albopictus* larvae (BOTH INF). Each treatment was replicated five times.

For the oocyst extraction in this experiment, approximately 400 adults of each species were macerated in 10 ml of distilled H_2O in a glass grinder. After grinding, 6 ml were added to the grinder to dilute the extract and then it was filtered through cotton in a glass syringe. Oocysts were counted with a haemocytometer. Counting was repeated four times and means were calculated from these replicates. A stock suspension of 100,000 oocysts/ml was prepared for each gregarine species.

Experimental larvae were infected at 24-hour after hatching with oocysts from the crude extract of bodies of infected mosquitoes without centrifugation. Thirty healthy mosquitoes of each species were macerated in a glass grinder with 3 ml distilled H_2O, and then 12 ml of distilled H_2O were added. From this extract, 1 ml was taken and added to each cup in the control treatments (AEG H ALONE, ALBO H ALONE and BOTH H) containing larvae during the 24-hour exposure time.

At the end of the infection time, larvae of each treatment were washed twice with distilled H_2O in 3-liter enamel pans. Then they were placed again in a clean plastic 100-ml cup filled with distilled H_2O. The 30 plastic cups comprising the six treatments, each one replicated five times, were taken to the field and placed in thirty individual tires (UF Medical Entomology Laboratory hammock).

Research Design

A randomized complete block design was used with six different gregarine-mosquito species composition combinations as treatments and five replicates (tires) per treatment. In the hammock, 30 golf-cart tires were placed in shady sites and randomly arranged in 10 3-tire groups prior to placing the larvae into each tire. Treatments and replicates were randomly allocated to tires.

Tire Handling

Tires used in the experiment were previously heat-sterilized by exposure to 50°C for 24 hours. After sterilization, they were carefully washed with well water. One liter of tire water was added to each each tire before addition of experimental larvae. Thirty liters of this water were removed from old discarded tires that were exposed in the hammock for two months before the experiment. The water was the normal accumulation of rainwater in tires in the field. Before adding 1 liter into each tire, the water was frozen for 36 hours to kill any insects or arthropods present. Four days before placement of the larvae into each tire, 1 g of dry oak leaves were put on the water surface of each tire. Four days were allowed for leaves to decompose and to function as a natural substrate for larval food. Oak leaves were collected from the forest floor and dried at 80 °

C for 48 h before placement into tires. Each group of three tires was covered with a screened, pyramidal-shaped cage to avoid oviposition by wild mosquitoes.

Data Collection

Five days after adding larvae, tires were examined daily for pupae. Pupae were removed every day from tires, taken to the laboratory and placed individually with 10 ml distilled H_2O in plastic screened-cup vials labeled with pupation date, treatment, and replicate number. A wet cotton pad was placed over each vial as a water source for adults. Adult emergence and death was recorded daily. After death, the sex of each mosquito was recorded and the left wing removed with forceps. Wing length (mm) was measured with an ocular micrometer from the alular notch to the wing margin distal excluding the scales (Washburn et al. 1989).

Infection by gregarines was checked in 5 males and 5 females of each mosquito species emerged from each tire (replicate) for all treatments (500 mosquitoes). After the death of these mosquitoes, the left wing was removed from each individual and the body was squashed with a cover slip on a slide to determine with a phase-contrast microscope if there were oocysts in the body.

The maximum and minimum temperature was recorded every seven days in the hammock from June 1 until July 6, 2000. Throughout this study, the average daily temperature varied from 27.0 to 28.75 °C.

Wing-Length and Fecundity

To estimate the relationship between wing-length and fecundity for both *Ae. aegypti* and *Ae. albopictus*, as a requirement for computing the performance index lambda (λ'), I reared healthy larvae of each mosquito species at a density of 400 larvae/liter in an enamel pan, and fed them with a regular diet (a 1:1 alfalfa: pigchow mix) used to maintain mosquito colonies in the Biological Control Laboratory at the CEMAVE. Larvae were fed 3 ml of a 5% larval diet added to each pan at approximately 48 hours (2nd day) after hatching, and additional 5 ml of the same suspension added to each pan at approximately 120 hours (5th day) post-hatching. Adults of each species were held in separate cages where they took a blood meal on a guinea pig. Twenty-four hours after blood feeding, 50 mosquitoes of each species were removed from the cage and individually separated in plastic screened-cap vials with a small piece of germination paper for oviposition plus 10 ml of distilled H_2O. After the first egg laying (1st gonotrophic cycle), 30 females were randomly chosen from the 50, and wing-length was measured as above described (Washburn et al. 1989). Linear regression analysis by the PROG REG procedure in SAS was conducted between wing-length and number of eggs laid as dependent variable for each species. Females were not dissected to observe unlaid eggs. Only the eggs laid by each female on the paper were included in the calculations.

Data Analysis

Data were analyzed with SAS procedures (SAS Institute 1988). The PROC MEANS procedure was used to estimate means from the five replicates for each treatment. Normality of raw data was analyzed by the option plot and the PROC UNIVARIATE procedure. PROC GLM and PROC ESTIMATE were used for analysis of variance and planned comparisons (apriori mean comparisons). Orthogonal contrasts were used because they permitted comparisons of combinations of means (Sokal and Rohlf 1969). The apriori mean comparisons carried out were three contrasts: Infected versus non-infected larvae of *Ae. aegypti*, infected versus non-infected larvae of *Ae. albopictus*, and intra- versus interspecific competition when all larvae were healthy.

For instance, to see effects by parasitism on *Ae. aegypti*, the pairs of means (using numbers of treatments from page 49) were combined for comparisons as:

(Mean # 1 + mean # 4)/2 vs (mean # 3 + mean # 5)/2.

Separate contrasts were performed for each variable included in the study: wing-length, immature development time, survivorship to adulthood, days as adult, and lambda (λ'), which is a composite performance index.

Lambda (λ')

Performance of each species also was evaluated by the estimated finite rate of increase (λ'), a population composite index derived from r' (λ' = exp r') of Livdahl and Sugihara (1984). This measurement is a robust estimate of the per capita rate of change (r) when experimental populations are established as cohorts, and fecundity and mortality data are available. Thus, the net reproductive value (R_o), which is the expected number of offspring per newborn individual, may be estimated from knowledge of the fraction of the original cohort maturing on day x and the future net fecundity of that fraction. The estimation of lambda may use the fecundity-body size relationship for the experimental species. So, linear regression equations were used to estimate the parameter *f(Wx),* which is required to compute the population composite index or lambda (exp (r')) (Livdahl and Sugihara 1984, Juliano 1998). The equation for λ' (= exp (r')) which unlike r' is estimable even with zero survival (Juliano 1998), is the following:

$$\lambda' = \exp\left[\frac{\ln\left[1/No\right]\sum_x Axf(Wx)}{D + \left[\frac{\sum_x xAxf(Wx)}{\sum_x Axf(Wx)}\right]}\right]$$

Where:

r' = composite index of performance

No = initial number of females in the cohort (assumed 50%)

Ax = number of females eclosed on day x, measured for species by replicate

Wx = Measure of the mean size of females eclosing on day x, measured for species by replicate

f(Wx)= function relating production of female eggs to female wing-length

D = Time between adult eclosion and reproduction (here I used 10 days for both species, which is the approximate time that I observed during the handling of my colonies).

Results

There was 100% of infection in the three infected treatments (AEG INF /ALBO H, AEG H /ALBO INF, and BOTH INF). There was not a mosquito positive for oocysts among the two hundred mosquitoes squashed in the control treatments: 25 males and 25 females of *Ae. aegypti* from the treatment AEG H ALONE, the same numbers per sex in the treatment ALBO H ALONE, and 100 (25 males and 25 females of *Ae. aegypti* plus the same numbers of *Ae. albopictus*) of both species from the treatment BOTH H.

Wing-Length - Fecundity Relationship

The linear regression equation relating fecundity and wing-length in healthy mosquitoes at the 1st gonotrophic cycle, was highly significant for both *Ae. aegypti* (F= 12.65, df = 29, $p<0.001$) and *Ae. albopictus* (F= 13.63, df = 29, $p<0.0001$) (Fig. 4-1, 4-2). However, the equation for *Ae. albopictus* ($R^2 = 0.52$) was a better predictor of the relationship for *Ae. aegypti* ($R^2= 0.31$).

Wing-Length

Wing-length was unaffected by treatments for either sex of the mosquito species. The F statistic in the four ANOVAs for wing-length (mm) carried out separately by sex in *Ae. aegypti* and *Ae. albopictus,* was not significant among treatments. This trend was also detected in the *t* statistic of the planned comparison analyses performed to estimate the effect of infection within the same species (infected versus healthy mosquitoes), and the impact of intra- versus interspecific competition when all larvae were healthy upon wing-length of mosquitoes emerged from the five treatments (Table 4–3, 4-4). Mean wing-length for *Ae aegypti* females and males fluctuated from 2.52 to 2.63 mm and from 1.97 to 2.06 mm respectively, while for *Ae. albopictus* females and males varied from 2.38 to 2.53 mm and from 1.91 to 1.98 mm respectively (Table 4–1, 4-2).

Immature Development Time

The immature development time in *Ae aegypti* did not differ among treatments (Table 4-3). In contrast, there were significant differences among treatments when the analysis was conducted for *Ae. albopictus* males. In the contrasts performed with planned comparisons for treatment means, the contrasts "healthy versus infected *Ae. aegypti* " and "intra- versus interspecific competition" were significant (Table 4-4). This occurred because *Ae. albopictus* males developed faster in the presence of infected *Ae. aegypti* larvae (12.05 d) than in the presence of healthy ones (12.87 d) (Table 4-2). In addition, the male development was significantly shorter under intraspecific (11.11 d) than under interspecific competition (13.03 d) (Table 4-2).

Survivorship to Adulthood

Despite a non-significant F-value for an effect of treatments, the contrast "healthy versus infected *Ae. aegypti*" was significant for *Ae. aegypti* because survivorship to adulthood for both sexes of *Ae aegypti* was significantly higher in the infected larvae (33.5%) than in healthy ones (25%) (Table 4-1). Specifically, the percent of larvae that survived to the adult stage was higher in the infected larvae (33% in the ALBO H / AEG INF, and 34% in the BOTH INF) than in the uninfected ones (24% in the ALBO INF / AEG H, and 26% in the BOTH H) (Table 4-1). For *Ae. albopictus*, neither the infection by *A. taiwanensis* nor the interspecific competition affected significantly the survivorship to adulthood (Table 4-4).

The Estimated Finite Rate of Increase or Lambda (λ')

Lambda values for *Ae. aegypti* were significantly different (Table 4-3) among treatments, but not for *Ae. albopictus* (Table 4-4). In *Ae. aegypti*, the contrast "intra- versus interspecific healthy" was significant because the index of performance for *Ae. aegypti* was higher under intra- (1.1588) than that under interspecific competition (1.1054). Performance of each mosquito species was not significantly affected by the parasitism treatments, i.e., the infection factors (Tables 4-3, 4-4).

Days as Adult

Days as adult varied significantly among treatments only for *Ae. albopictus* males. Only the contrast "intra- versus interspecific healthy" in the planned comparisons was significant, which means that the infection factor was not significant here (Table 4-4). The number of days as adult for *Ae. albopictus* males was low under intraspecific (ALBO ALONE) competition (3.13 d) than under interspecific (BOTH H) competition (3.87 d) (Table 4-2). There were no significant differences for *Ae. aegypti*, in which the days as adult fluctuated from 4.24 d to 4.88 d for females, and from 4.57 to 5.05 d for males (Table 4-1).

Discussion

The regression equations predicted the relationship between body size and fecundity for both mosquito species. However, it is necessary to clarify that these equations were generated from experimental larvae held at a middle density (400 larvae/liter), which means not overcrowded. In addition, the larval diet used was a high nutrient diet. These equations are much better in predictive power that others published in the literature. In a recent paper on *Ae. albopictus*, the r^2 values for the equation relating wing-length with eggs laid per female was low (0.07), and many methodological details are missing in the paper such as the actual number of larvae reared per liter, and how many females per larval density were included in the calculation (Blackmore and Lord 2000). Although I did not dissect the females for unlaid eggs, I think the equations calculated in this study are acceptable to be used in field population dynamics in both *Ae. aegypti* and *Ae. albopictus*.

In the interspecific competition, the development time of *Ae. albopictus* males was shortened in the presence of infected *Ae. aegypti* compared to uninfected larvae of this species. Perhaps *A. culicis* is able to cause some deleterious effect upon *Ae. aegypti* larvae and sick larvae have a lower feeding rate than the uninfected. Statistical analysis of data did not show significant differences in competitionThe potential advantage for *Ae. albopictus* because of its shorter larval development in males, could be compensated by a higher larval survivorship to adulthood (both sexes) exhibited by *Ae. aegypti* larvae infected by *A. culicis*.

Juliano (1998) found in *Ae. albopictus* larvae a parasitism by gamonts that he assumed were *A. taiwanensis*. He concluded that *A. taiwanensis* really had no effect on

Ae. albopictus when it was competing with *Ae. aegypti* because he virtually did not found gamonts in *Ae. aegypti* larvae.

Perhaps the gregarines are benign parasites that do not exert a negative impact on their hosts when both compete under unstressful conditions. In my study, although the infection was relatively high (1000 oocysts/larva), the larval density of 100 larvae/liter in treatments is a low larval density (no overcrowding) (Clements 1992) and natural food from 1 g of oak leaves could provide a nutritional level sufficient for the larvae to tolerate the gregarine infection without a significant negative impact.

This study was exploratory. It is the first conducted to evaluate the role of *Ascogregarina* species in larval competition between *Ae. aegypti* and *Ae. albopictus* in the field by controlling the infection factor (1000 oocysts/larva). In this experiment, larvae of each mosquito species were infected with its own *Ascogregarina* species (*Ae. aegypti* with *A. culicis* and *Ae. albopictus* with *A. taiwanensis*) and confronted one each other in tires, without cross and dual infections. At least at the dose of 1000 oocysts/larva neither *A. culicis* nor *A. taiwanensis* was able to reduce significantly the fitness of their corresponding mosquito hosts when they competed in the field. Studies involving higher larval densities (larval overcrowding) and cross and dual infections to set up more stressful conditions on experimental larvae may be useful. In this study, the recently hatched larvae were fed with brewer's yeast and they were infected at 24 h after hatching. The first 24 h after hatching is critical to survive in mosquito larvae attacked by gregarines because in that time the eight sporozoites released from each oocyst in larval midgut become intracellular parasites in the epithelium (McCray et al. 1970). I think that the brewer's yeast provided as food to the larvae during the first 24 h, allowed them to survive the infection by gregarines because of their good healthiness. Larvae were exposed to the oocysts as 24-h old larvae. It is necessary to provide natural food (as oak dry leave infusion) to the recently hatched larvae that comprise the treatments in competition experiments. Perhaps, under strong stressful conditions, the gregarines are able to impact negatively the fitness of their host and therefore affect the competitive interaction between both mosquito species. Whether the gregarines have a role mediating the competition between these two sympatric mosquito species may only be observed when competitors encounter more stressful conditions in the field.

Table 4-1. Mean ± SE (Standard Error) of wing-length (mm), immature development time (days), survival to adulthood (days) (pooled sexes), lambda Index (λ') (pooled sexes) and days as adult for *Aedes aegypti* emerged from mixed larval treatments (with *Ae. albopictus*) and from single larval treatments[1] placed into tires in field, and each mosquito host infected with its own *Ascogregarina* species at 1000 oocysts/larva.

	Treatments									
	ALBO H / AEG INF		ALBO INF / AEG H		BOTH H		BOTH INF		AEG ALONE	
Variable	F[2]	M[3]	F	M	F	M	F	M	F	M
Wing-Length	2.56 ± 0.04	2.00 ± 0.04	2.63 ± 0.04	2.06 ± 0.03	2.52 ± 0.07	2.03 ± 0.03	2.60 ± 0.04	2.03 ± 0.03	2.59 ± 0.04	1.97 ± 0.04
Immature Development Time	16.11 ± 0.88	13.12 ± 0.66	14.63 ± 0.56	11.87 ± 0.55	15.50 ± 1.33	12.56 ± 0.70	13.90 ± 0.86	11.25 ± 0.66	15.50 ± 1.44	12.74 ± 0.87
Survival to Adulthood	0.33 ± 0.03		0.24 ± 0.04		0.26 ± 0.05		0.34 ± 0.03		0.30 ± 0.03	
Lambda (λ')	1.1158 ± 0.0095		1.1081 ± 0.0050		1.1054 ± 0.0188		1.1285 ± 0.0065		1.1588 ± 0.0146	
Days as Adult	4.45 ± 0.32	4.74 ± 0.55±	4.88 ± 0.04	5.05 ± 0.16	4.37 ± 0.15	4.71 ± 0.37	4.24 ± 0.17	4.84 ± 0.41	4.31 ± 0.15	4.57 ± 0.19

[1] ALBO H / AEG INF = *Ae. albopictus* healthy/*Ae. aegypti* infected, ALBO INF / AEG H = *Ae. albopictus* infected / *Ae aegypti* healthy, BOTH H = Both species healthy, BOTH INF = Both species infected, AEG ALONE = Only *Ae. aegypti* uninfected.

[2] Female

[3] Male

Table 4-2. Mean ± SE (Standard Error) of wing-length (mm), immature development time (days), survival to adulthood (%) (pooled sexes), lambda Index (λ') (pooled sexes) and days as adult for *Aedes albopictus* emerged from mixed larval treatments (with *Ae. aegypti*) and from single larval treatments[1] placed into tires in field, and each mosquito host infected with its own *Ascogregarina* species at 1000 oocysts/larva.

Variable	Treatments									
	ALBO H / AEG INF		ALBO INF / AEG H		BOTH H		BOTH INF		ALBO ALONE	
	F[2]	M[3]	F	M	F	M	F	M	F	M
Wing-Length	2.38 ± 0.05	1.91 ± 0.03	2.53 ± 0.04	1.98 ± 0.02	2.47 ± 0.06	1.91 ± 0.06	2.48 ± 0.04	1.98 ± 0.05	2.49 ± 0.01	1.92 ± 0.05
Immature Development Time	16.90 ± 0.70	12.05 ± 0.58	16.69 ± 0.26	12.87 ± 0.27	18.00 ± 0.94	13.03 ± 0.53	14.77 ± 1.48	10.62 ± 0.60	16.14 ± 1.30	11.11 ± 0.39
Survival to Adulthood	0.27 ± 0.04		0.25 ± 0.03		0.30 ± 0.04		0.26 ± 0.04		0.26 ± 0.01	
Lambda (λ')	1.1207 ± 0.0079		1.1268 ± 0.0055		1.1245 ± 0.0133		1.1348 ± 0.0106		1.1300 ± 0.0064	
Days as Adult	4.26 ± 0.54	3.53 ± 0.46	4.24 ± 0.19	4.90 ± 0.21	4.21 ± 0.46	3.87 ± 0.45	4.25 ± 0.33	3.30 ± 0.33	4.11 ± 0.36	3.13 ± 0.19

[1] ALBO H / AEG INF = *Ae. albopictus* healthy/*Ae. aegypti* infected, ALBO INF / AEG H = *Ae. albopictus* infected /*Ae. aegypti* healthy, BOTH H = Both species healthy, BOTH INF = Both species infected, ALBO ALONE = Only *Ae. albopictus* uninfected.

[2] Female

[3] Male

Table 4-3. Summary of ANOVAs and Planned Comparisons* (SAS Institute 1988) of treatment means for effects on univariate measures and composite index of performance (lambda) for *Aedes aegypti.*

		Immature Development Time (days)		Wing-length (mm)		Immature Survival (%)	Lambda	Adult Survivorship (days)	
Source	df	Males	Females	Males	Females	(Both sexes)	(Both sexes)	Males	Females
Treatment[1]	4	1.16	0.63	0.60	0.70	1.07	3.25*	0.28	1.73
SS Error[2]	20	0.9154	1.8030	0.1938	0.2586	0.1832	0.0146	0.6375	0.2116
Contrasts									
1 (Healthy vs Infected *Ae. albopictus*)		1.84	1.40	-1.00	-1.47	0.10	-0.64	-0.76	-0.81
2 (Healthy vs Infected *Ae. aegypti*)		0.02	0	0.50	-0.11	-2.04*	-1.27	0.37	1.57
3 (Intra vs Interspecific healthy)		-0.20	-0.02	0.52	-1.03	-0.67	-3.12*	0.20	0.18

[1] The row comprises the F values of the ANOVA conducted for each variable.

[2] Square Sum of Error of each ANOVA.

* Significant F and student-t values ($p < 0.05$).

Table 4-4. Summary of ANOVAs and Planned Comparisons* (SAS Institute 1988) of treatment means for effects on univariate measures and composite index of performance (lambda) for *Aedes albopictus.*

		Immature Development Time (days)		Wing-length (mm)		Survival to Adulthood (%)	Lambda	Days as Adult	
Source	df	Males	Females	Males	Females	(Both sexes)	(Both sexes)	Males	Females
Treatment[1]	4	4.54*	1.38	0.63	1.18	0.27	0.33	3.51*	0.02
SS Error[2]	20	0.4819	1.6523	0.2373	0.2504	0.1577	0.0085	0.8674	0.8440
Contrasts									
1 (Healthy vs Infected *Ae. albopictus*)		1.59	1.66	-1.53	-1.63	0.79	-0.88	-1.01	-0.09
2 (Healthy vs Infected *Ae. aegypti*)		3.23*	1.54	0.03	1.29	0.26	-0.23	2.57*	-0.03
3 (Intra- vs Interspecific healthy)		2.62*	1.28	-0.19	-0.36	0.73	-0.42	1.43	0.15

[1] The row comprises the F values of the ANOVA conducted for each variable.

[2] Square Sum of Error of each ANOVA.

* Significant F and student-t values ($p < 0.05$).

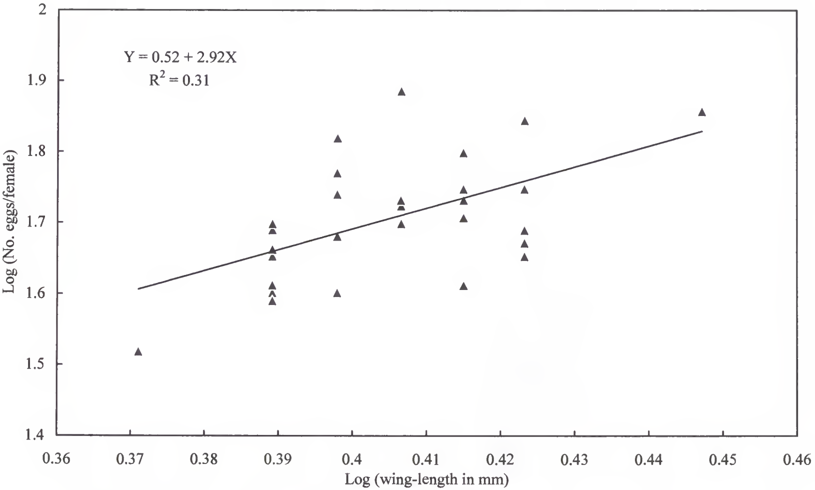

Fig. 4-1. Linear regression of number of eggs deposited at the first gonotrophic cycle against wing-length (mm) in a sample of *Aedes aegypti* healthy females (Tampa strain).

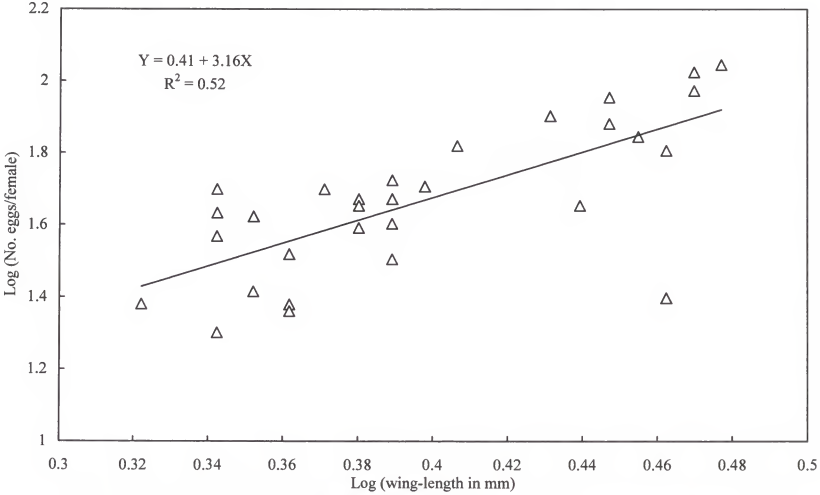

Fig. 4-2. Linear regression of number of eggs deposited at the first gonotrophic cycle against wing-length (mm) in a sample of *Aedes albopictus* healthy females (Tampa strain).

CHAPTER 5
SUMMARY AND CONCLUSIONS

Four experiments were conducted under laboratory conditions and a fifth one in the field to determine the impact of the gregarines *Ascogregarina culicis* and *A. taiwanensis* upon the biology and fitness of their respective hosts, the mosquitoes *Aedes aegypti* and *Ae. albopictus*. The general purpose of this study was to determine host/parasite relationships in these host-specific gregarines often considered as non-deleterious parasites on their normal hosts (Beier and Craig 1985) and to use this information to explore whether they may mediate the outcome of competition between *Ae. aegypti* and *Ae. albopictus*. Therefore, direct and indirect effects by gregarines upon their hosts may change the competitive interaction between both mosquitoes in comparison with a scenario in which both are gregarine-free competitors.

Experiments were performed with wild strains of both host and parasite collected in Tampa, FL where prevalence of both gregarines is high (Blackmore et al. 1995) in mosquito populations. In addition, competition between both *Aedes* species is in progress in that locality with a displacement of *Ae. aegypti* by *Ae. albopictus*, as has already occurred in North Florida(O'Meara et al. pers. comm).

I demonstrated that *Ae. aegypti* is more readily parasitized by its own gregarine *A. culicis*, than *Ae. albopictus* is parasitized by *A. taiwanensis*. Larval-pupal mortality rate caused by both parasites upon their hosts was not significantly different in the two host/parasite systems. Infection did not affect the pupation rate of either host.

Prior to this study, there was not enough information in the literature to distinguish gamonts of *A. culicis* from those of *A. taiwanensis* in dual-infected larvae of either mosquito species. We found that *A. culicis* gamonts at approximately 6 hours before pupation are characterized by a brown pigmentation in the cytoplasm and a tapered protomerite, while those of *A. taiwanensis* are translucent and have a rounded protomerite.

At an initial infection rate of 100 oocysts/larva in single and dual infections, *Ae. aegypti* harbored a gamont intensity per larva of *A. culicis* that was twice as high as that observed in *Ae. albopictus* by *A. taiwanensis*. Each gregarine is specific for its natural mosquito host.

Two larval food levels (complete regular diet versus 75% reduced regular diet) and a high infection level (1000 oocysts/larva) were evaluated for effects upon wing-length and larval development time. Effects by parasitism were minimal and only reflected by a reduction in size of *Ae. aegypti* males from undernourished and infected larvae. In the case of larval development time, parasitism exerted a differential impact upon the sex of the host species. *A. culicis* increased larval development of *Ae. aegypti* males but not in females in the well fed larvae. Mixed exposures to *A. culicis* and *A. taiwanensis* increased larval development time in *Ae. aegypti* females and *Ae. albopictus* males (Table 3-5).

Finally, in the field experiment that comprised six treatments (4 with two species of larvae and 2 with one species of larvae) set up with infections by one parasite species (1000 oocysts/larva) and uninfected (controls), and intra- versus interspecific competition factors were tested by placing the treated larvae in tires. *Ae. albopictus* males had a shorter larval development time in the presence of infected *Ae. aegypti*. A higher survivorship to adulthood was exhibited by *Ae. aegypti* infected with *A. culicis* compared to non-infected controls. Wing-length and the performance index (lambda) in both competitors were not affected by the gregarine parasitism, although uninfected *Ae. aegypti* had a better performance, measured by lambda index under intra- than in interspecific competition.

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Filiberto Reyes-Villanueva was born in Monterrey, Nuevo Leon, Mexico, on August 22, 1951. He graduated from Preparatoria No. 8 (= High School) in 1968 and entered the Facultad de Ciencias Biologicas at Universidad Autonoma de Nuevo Leon in 1969. As undergraduate, he wrote a thesis titled "Insect Parasites of the Fall Webworm, *Hyphantria cunea* in some areas of the Nuevo Leon State" in 1974. He received the title of Biologist as a result of this study. He obtained the Master of Science Degree in Entomology from the Colegio Superior de Agricultura Tropical (Mexican Government) in 1977 with a thesis research on "The Role of Ants in the Biological Control of the Black Aphid, *Toxoptera aurantii* in the Cultivated Cocoa from Tabasco, Mexico", under the coadvisory of Dr. Willard H. Whitcomb, Visiting Professor.

After graduating with the Master of Science degree he was appointed to the position as Professor of Entomology in the Facultad de Ciencias Biologicas of the Universidad Autonoma de Nuevo Leon, in Monterrey, Mexico in 1978. From 1978 through 1997, he taught Entomology and worked as a Research Entomologist. In 1992, he took a sabbatical year as visiting researcher to study Onchocerciasis entomology in the Colegio de la Frontera Sur, former Centro de Investigaciones Ecologicas del Sureste (Mexican Government). He has been a member since 1987 (National Researcher level I) of the Sistema Nacional de Investigadores of Mexico (CONACyT - Federal Government). The focus of his research in the last thirteen years has been on Vector Biology and

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

L. Philip Lounibos

L. Philip Lounibos, Cochair
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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August 2001

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